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ON THE RELATIONS BETWEEN THE DISTRIBUTION OF SEA WATER TEMPERATURE, WIND-STRESS-CURL, UPWELLING AND SINKING.

Yasukazu SAITO and Seichi KANARI

(Received Feb. 5, 1960)

I. Introduction

When we will draw the distribution of sea water temperature in a certain sea region, we need some data of sea water temperature which were observed at each stations in the same region. But, generally, oceanic data are very poor in comparison with the meteorological data. Accordingly, if we can know the relations between sea water temperature and meteorological factors, it is possible to draw the pattern of distribution of sea water temperature by making use of the meteorological date.

In this paper, we will treat this problem under the following consideration. Namely, the sea water temperature pattern may be influenced by the ocean current caused by the wind stress in the same region under consideration. Therefore, if we can obtain the equation which expresses directly the relation between the wind stress and the temperature of sea water, we will be able to get the picture of the distributions of sea water temperature by means of the given meteorological data, solving this equation.

In the section of the theoretical treatment, the relations between the distributions of sea water temperature, wind stress curl, vorticity, upwelling and sinking will be discussed. In this case, the effect of the latitude variation of the Corioli's parameter is neglected, because the region of the ocean considered is a small scale. And also the effects of the enertial and the horizontal mixing terms are neglected.

It will be shown that the equation deducted is reasonable, using the data of sea water temperature and wind stress curl in the Gulf of Mexico as a numerical example

2. The deduction of the basic equation

In the case of our treatment, the condition of ocean current is assumed to be steady state. Then, the equations of motion in the small scale sea region are as follows.

$$\rho l v - \frac{\partial p}{\partial x} + \frac{\partial}{\partial z}\left[A_z(z) \frac{\partial u}{\partial z} \right] = 0 \qquad (1)$$

$$-\rho l u - \frac{\partial p}{\partial y} + \frac{\partial}{\partial z}\left[A_z(z) \frac{\partial v}{\partial z} \right] = 0 \qquad (2)$$

The equation of continuity is

$$\frac{\partial(\rho u)}{\partial x}+\frac{\partial(\rho v)}{\partial y}+\frac{\partial(\rho w)}{\partial z}=0 \qquad (3)$$

where, ρ=the density of sea waters.
l =the Corioli's parameter.
(φ=latitude).
(ω=angular velocity of the earth's rotation).
$A_z(z)$=coefficient of vertical mixing in the direction of z-axis.
(z-axis is directed vertically downwards).
u=velocity in the direction of x-axis.
(x-axis is directed eastwards).
v=velocity in the direction of y-axis.
(y-axis is directed northwards).
w=velocity in the direction of z-axis.
p=pressure.

Then, by operating the cross-differentiation to the equations (1) and (2), and subtracting the results, we obtain the following formula,

$$-l\left\{\frac{\partial(\rho u)}{\partial x}+\frac{\partial(\rho v)}{\partial y}\right\}+\frac{\partial}{\partial z}\left[A_z(z)\frac{\partial}{\partial z}\left(\frac{\partial v}{\partial x}-\frac{\partial u}{\partial y}\right)\right]=0,$$

and from (3), it follows that

$$-l\left\{\frac{\partial(\rho w)}{\partial z}\right\}=\frac{\partial}{\partial z}\left[A_z(z)\frac{\partial}{\partial z}\left(\frac{\partial v}{\partial x}-\frac{\partial u}{\partial x}\right)\right]$$

Therefore, integrating from z to h, we have

$$w_{(z)}=-\frac{A_z(z)}{lp}\cdot\frac{\partial}{\partial z}\left(\frac{\partial v}{\partial x}-\frac{\partial u}{\partial y}\right) \qquad \text{at } z\text{-layer,}$$

where

$$w\Big|_{z=h}=0, \qquad A_z\frac{\partial v}{\partial z}\Big|_{z=h}=0, \qquad A_z\frac{\partial u}{\partial z}\Big|_{z=h}=0.$$

Putting

$$\zeta=\frac{\partial v}{\partial x}-\frac{\partial u}{\partial y},$$

we have

$$w_{(z)}=-\frac{A_z(z)}{\rho l}\cdot\frac{\partial\zeta_{(z)}}{\partial z} \qquad (0<z<h) \qquad (4),$$

where $w_{(z)}$ and $\zeta_{(z)}$ mean the vertical current velocity and vorticity at z-layer, respectively.

Therefore,

$$\text{if} \quad \frac{\partial\zeta_{(z)}}{\partial z}>0, \qquad \text{then } w_{(z)}<0 \qquad (5),$$

$$\text{if} \quad \frac{\partial\zeta_{(z)}}{\partial z}<0, \qquad \text{then } w_{(z)}>0 \qquad (6)$$

Namely, the case of (5) means the upwelling and the case of (6) the sinking.

Accordingly, if we can know the value of the vorticity gradient in the direction of z-axis, the sighn of the vertical velocity will be determined. Therefore, by computing the value of the velocity, it will be possible to determine the region of sinking or upwelling. But, in the actual oceanographical survey, the data concerning the measurement of current velocity are very few. Therefore, it is difficult to obtain the value of ζ.

Accordingly, in order to detect this point, the following treatment will be considered.

The data of the measurement of the temperature of sea water are rich with the comparison of that of vorticity. Accordingly, it is necessary to get the relation between the temperature, vorticity, and vertical velocity.

First of all, as a first approximation, by means of the following treatment, we can obtain the values of the current velocity in the direction of the x and y axes respectively.

Namely,
$$u=-\frac{1}{l}\cdot\frac{\partial D}{\partial y}, \quad v=\frac{1}{l}\cdot\frac{\partial D}{\partial x} \tag{7}$$
where D means the dynamic depth.

Now, the above formula (7) is derived from the underying equation without considering the terms of the mixing.

$$\left.\begin{aligned} \rho lv-\frac{\partial p}{\partial x}&=0 \\ -\rho lu-\frac{\partial p}{\partial y}&=0 \end{aligned}\right\} \tag{8}$$

But our previous basic equations are

$$\left.\begin{aligned} \rho lv-\frac{\partial p}{\partial x}+\frac{\partial}{\partial z}\left[A_z(z)\frac{\partial u}{\partial z}\right]&=0 \\ -\rho lu-\frac{\partial p}{\partial y}+\frac{\partial}{\partial z}\left[A_z(z)\frac{\partial v}{\partial z}\right]&=0 \end{aligned}\right\} \tag{9}$$

Namely, the equation (9) includes the term of the vertical mixing Therefore, it will be not necessary self-consistent to treat the equations (8) and (9) simultaneously. But, by giving the following consideration to this treatment, we can detect the non-self-consistency mentioned above.

According to the experience which has been so far get, it is usually admitted that the values of current velocity obtained by using the methode of the dynamical computation are comparatively equal to the actual observed values of current velocity. Therefore, we will use the formula (7) by regarding as the semi-empirical formula.

Now,
$$D=\int_z^h \alpha dz \qquad (h \text{ means the depth of no current layer.})$$
where α means the specific volume of the sea waters. Therefore, neglecting the dimensional difference, we have
$$dD=\alpha dp\approx\alpha dz \tag{10}$$

where the unit of p is deciber and z meter. Though the dimensional unit in the both sides of the formula (10) are different, the numerical equality will be held because of the following numerical relation

$$1 \text{ (deciber}=10^5 \text{ dyne/cm}^2)\approx 1 \text{ (meter}=10^2 \text{ cm)}.$$

Then, in order to make the dimension in the latter terms of (10) equal to that in the former, the following coefficient η will be introduced

$$dD=\alpha dp\approx\eta\alpha dz \tag{11}$$

where, $\eta=10^3$ dyne/cm^3.

Furthermore, we will assume that the variation of the numerical values of specific volume is proportional to that of water temperature, in the certain domain of the values of α. Namely, $\alpha\propto\theta$, where θ means the sea water temperature.

Accordingly, $$\alpha=\gamma\theta+c \tag{12}$$

where γ means the constant coefficient with the same dimension as α, and C the specific volume in the case $\theta=0$.
By using this relation, we will get the following formula.

$$dD=\alpha dp\approx\eta\cdot\alpha dz\approx\eta\cdot(\gamma\theta+c)dz$$

Therefore, we have

$$D=\eta\int_z^h(\gamma\theta+c)dz=\int_z^h(\gamma'\theta+\eta c)dz\,, \qquad \text{where } \gamma'=\eta\gamma\,.$$

Then, from (7) we obtain the following equations:

$$u=-\frac{1}{l}\frac{\partial D}{\partial y}\approx-\frac{\gamma'}{l}\int_z^h\frac{\partial\theta}{\partial y}dz \tag{13}$$

$$v=\frac{1}{l}\cdot\frac{\partial D}{\partial y}\approx\frac{\gamma'}{l}\int_z^h\frac{\partial\theta}{\partial z}dz \tag{14}$$

where, we considered as $\frac{\partial c}{\partial x}\approx 0$ $\frac{\partial c}{\partial y}\approx 0$ in the deeper layer.

From (13) and (14) we obtain the following formula.

$$\zeta=\frac{\partial v}{\partial x}-\frac{\partial u}{\partial y}\approx\frac{\gamma'}{l}\int_z^h\left(\frac{\partial^2\theta}{\partial x^2}+\frac{\partial^2\theta}{\partial y^2}\right)dz \tag{15}$$

Accordingly,

$$\frac{\partial\zeta}{\partial x}\approx-\left\{\frac{\gamma'}{l}\left(\frac{\partial^2\theta}{\partial x^2}+\frac{\partial^2\theta}{\partial y^2}\right)\right\}=-\frac{\gamma'}{l}(\nabla^2\theta \qquad \text{at } z\text{-layer} \tag{16}$$

where $0<z<h$, and

$$\nabla^2=\frac{\partial^2}{\partial x^2}+\frac{\partial^2}{\partial y^2}$$

From (15),

$$\zeta=\frac{\gamma'}{l}\int_z^h\nabla^2\theta dz=\frac{\gamma'}{l}\nabla^2[\int_z^h\theta dz]=\frac{\gamma'}{l}\nabla^2\Theta \tag{17}$$

where

$$\Theta=\int_z^h\theta dz\ .$$

Using the formule (4) and (16), we obtain the following equation.

$$w\approx\frac{A_z(z)\gamma'}{\rho l^2}\nabla^2\theta \tag{18}$$

Let $w_{(z)}$ and $\theta_{(z)}$ denote respectively the vertical current velocity and the sea water temperature at z-layer, the formula (18) follows that

$$w_{(z)}\approx\frac{A_z(z)\gamma'}{\rho l^2}\nabla^2\theta_{(z)} \qquad (0<z<h). \tag{19}$$

Therefore, from (19), we have the following relations.

$$\text{If}\quad \nabla^2\theta_{(z)}>0,\ \text{then}\ w_{(z)}>0\ . \tag{20}$$

$$\text{If}\quad \nabla^2\theta_{(z)}<0,\ \text{then}\ w_{(z)}<0\ . \tag{21}$$

where the case of (20) means the sinking and the case of (21) the upwelling. From (17), we also have the following relations.

$$\text{If}\quad \nabla^2\Theta>0,\ \text{then}\ \zeta>0\ . \tag{22}$$

$$\text{If}\quad \nabla^2\Theta<0,\ \text{then}\ \zeta<0\ . \tag{23}$$

Namely, evaluating the values of $\Delta^2\theta_{(z)}$ and $\Delta^2\Theta$ from the chart of the distributions of sea water temperature, the regions of positive and negative vorticity, upwellings and sinkings can be determined.

Next, let us consider the case in which the wind blows over the sea surface. Now, let $\tau_y(z)$ and $\tau_y(z)$ denote the components of vertical shearing stress at z-layer in the direction of x and y axes respectively. Then, the equations of motion under the steady state are as follows:

$$\rho lv-\frac{\partial p}{\partial x}-\frac{d\tau_{x(z)}}{dz}=0 \tag{24}$$

$$-\rho lu-\frac{\partial p}{\partial x}-\frac{d\tau_{y(z)}}{dz}=0 \tag{25}$$

where

$$-\tau_{x(z)}=A_z(z)\frac{\partial u}{\partial z} \qquad -\tau_{y(z)}=A_z(z)\frac{\theta v}{\partial z}\ .$$

And the boundary conditions are as follows.

$$\tau_{x(z)}\Big|_{z=0}=\tau_{x(0)}\ , \qquad \tau_{y(z)}\Big|_{z=0}=\tau_{y(0)} \tag{26}$$

$$\tau_{x(z)}\Big|_{z=h}=0\ , \qquad \tau_{y(z)}\Big|_{z=h}=0 \tag{27}$$

where, $\tau_{x(o)}$ and $\tau_{y(o)}$ mean the wind stress at the sea surface. And the equation of continuity is as follows:

$$\frac{\partial(\rho u)}{\partial x}+\frac{\partial(\rho v)}{\partial y}+\frac{\partial(\rho w)}{\partial z}=0 \tag{28}$$

By operating the cross-differentiation to the equations (24) and (25), and subtracting the result obtained, it follows that,

$$l\left\{\frac{\partial(\rho u)}{\partial x}+\frac{\partial(\rho u)}{dy}\right\}+\frac{\partial}{\partial z}\left[\frac{\partial\tau_{y(z)}}{\partial x}-\frac{\partial\tau_{x(z)}}{\partial y}\right]=0\,.$$

From (28), it follows that

$$\frac{\partial(\rho w)}{\partial z}=\frac{1}{l}\frac{\partial}{\partial z}\left[\frac{\partial\tau_{y(z)}}{\partial x}-\frac{\partial\tau_{x(z)}}{\partial y}\right] \tag{29}$$

Integrating (29) under the consideration of the conditions of (27) and $w_{(h)}=o$, we have the following equation

$$\begin{aligned} w&=\frac{1}{\rho l}\left[\frac{\partial\tau_{y(z)}}{\partial x}-\frac{\partial\tau_{x(z)}}{\partial y}\right] \\ &=\frac{1}{\rho l}C_{url}\,\tau \end{aligned} \tag{30}$$

Let $w_{(z)}$ and $\tau_{(z)}$ denote the vertical current velocity and current stress at z-layer depth respectively, it follows that

$$w_{(z)}=\frac{1}{\rho l}C_{url}\,\tau_{(z)} \qquad 0<z<h \tag{31}$$

Accordingly, we obtain the following relation:

if $C_{url}\,\tau_{(z)}>0$, then $w_{(z)}>0$ (32)

if $C_{url}\,\tau_{(z)}<0$, then $w_{(z)}<0$ (33)

where (32) means the sinking and (33) the upwelling.

Now, the previous equations (1) and (2) express the case of stady state of the motion of sea waters given rise up by any active sources. And in this case it is no matter that by what active source the motion of sea waters were caused.

Therefore, the formula $w_{(z)}=\frac{A_z(z)\gamma'}{\rho l^2}\cdot\nabla^2\theta_{(z)}$ introduced from these equations, also holds the same character.

Now, we will consider the case that the wind stress corresponds to the active source by which the motion of sea waters is caused.

Accordingly, it will be not unreasonable to combine $w_{(z)}=\frac{A_z(z)\gamma'}{\rho l^2}\cdot\nabla^2\theta_{(z)}$ with $w_{(z)}=\frac{1}{\rho l}C_{url}\,\tau_{(z)}$.

Then, combining these two formulae, we obtain the following equation:

$$\frac{A_z(z)\gamma'}{l}\cdot\nabla^2\theta_{(z)}=C_{url}\,\tau_{(z)} \qquad (0<z<h) \tag{34}$$

Putting

$$K_p(z)=\frac{A_z(z)\gamma'}{l}=\frac{A_z(z)\gamma'}{2\omega\sin\varphi}\,,$$

we have

$$K_p(z)\nabla^2\theta_{(z)}=Curl\,\tau_{(z)} \qquad \text{at } z \text{ layer} \qquad (35)$$

From the above equation, we get

$$K_p(o)\nabla^2\theta_{(o)}=Curl\,\tau_{(o)} \qquad \text{with } z\to 0 \qquad (36)$$

as approching to the sea surface.

Where,

$$Curl\,\tau_{(o)}=\frac{\partial\tau_{y(o)}}{\partial x}-\frac{\partial\tau_{x(o)}}{\partial y},$$

(this means wind stress curl at the sea surface.)
$\theta_{(o)}$ means

$$\theta_{(o)}=\theta\,|_{z=0}=\theta(x, y, o),$$

this means the sea surface temperature.

$$K_{p(0)}=\frac{1}{2\omega\sin\varphi}\cdot A_z(o)\gamma'$$

The dimension of $K_p(z)$ is as follows:

$$[\gamma]=[\alpha]=\frac{[L^3]}{[M]}, \qquad [\eta]=\frac{[M]}{[T^2][L^2]}.$$

Therefore,

$$[\gamma']=[\gamma]\cdot[\eta]=\frac{[L]}{[T^2]},$$

and

$$[A_z]=\frac{[M]}{[L][T]}, \qquad [W]=\left[\frac{1}{T}\right]$$

Then,

$$[K_p]=\left[\frac{M}{T^2}\right]=\left[\frac{M}{LT}\right]\cdot\left[\frac{L}{T}\right]$$

$$=[\text{Coefficient of Vertical Austauschi}]\cdot[\text{Velocity}].$$

Accordingly, it seems that coefficient $K_p(z)$ plausibly corresponds to the role of the advection of the horizontal mixing. Therefore, we wish to name this constant $K_p(z)$ as the plausible horizontal mixing coefficient.

3. Numerical Example.

In the previous paragraph, the relations between the distributions of wind stress, sea water temperature, and vorticity under the several assumptions were introduced. In this paragraph, let us discuss the validity of the previous relations by mean of the practical data. Though the equation (35) $K_{p(z)}\Delta^2\theta_{(z)}=\text{Curl}\,\tau_{(z)}$ is applicable to the layer at any depth, only the data of sea water temperature and wind stress at the sea surface are given in this paper. Therefore, we will discuss only the case of the equation (36) $K_{p(0)}\Delta^2\theta_{(0)}=\text{Curl}\,\tau_{(0)}$, namely the case of sea surface. And also the possibility of the assumption of $\alpha_{s.\theta.z}=\gamma\theta_{(z)}+\text{C}$ will be tested.

The data used here are as follows.

(1) The distribution of the mean monthly wind stress over the Gulf of Mexico.

by Guy A. Franceschini.
(The A & M. College of Texas, Department of Oceanography).
Scientific Report No. 1. August, 1953.

(2) Physical Oceanography of the Gulf of Mexico.
by Dale F. Leipper.
Gulf of Mexico Its Origin, Waters, and Marine Life.
Fishery Bulletin 89 (p. 119).
Fishery Bulletin of the Fish and Wildlife Service. vol. 55, 1954.

The computation of Curl τ.

The value of Curl τ at the sea surface were computed from the data (1) which consist of tabulations of the areal distribution of the mean monthly and annual wind stress, sea surface temperature, and current velocity obtained from ship observations for the period 1855~1950. These tabulations of wind stress also include the wind direction to 8 points.

We will use only the data in December because of the absence of sea water temperature data in other months. Now, the mean distributions of wind

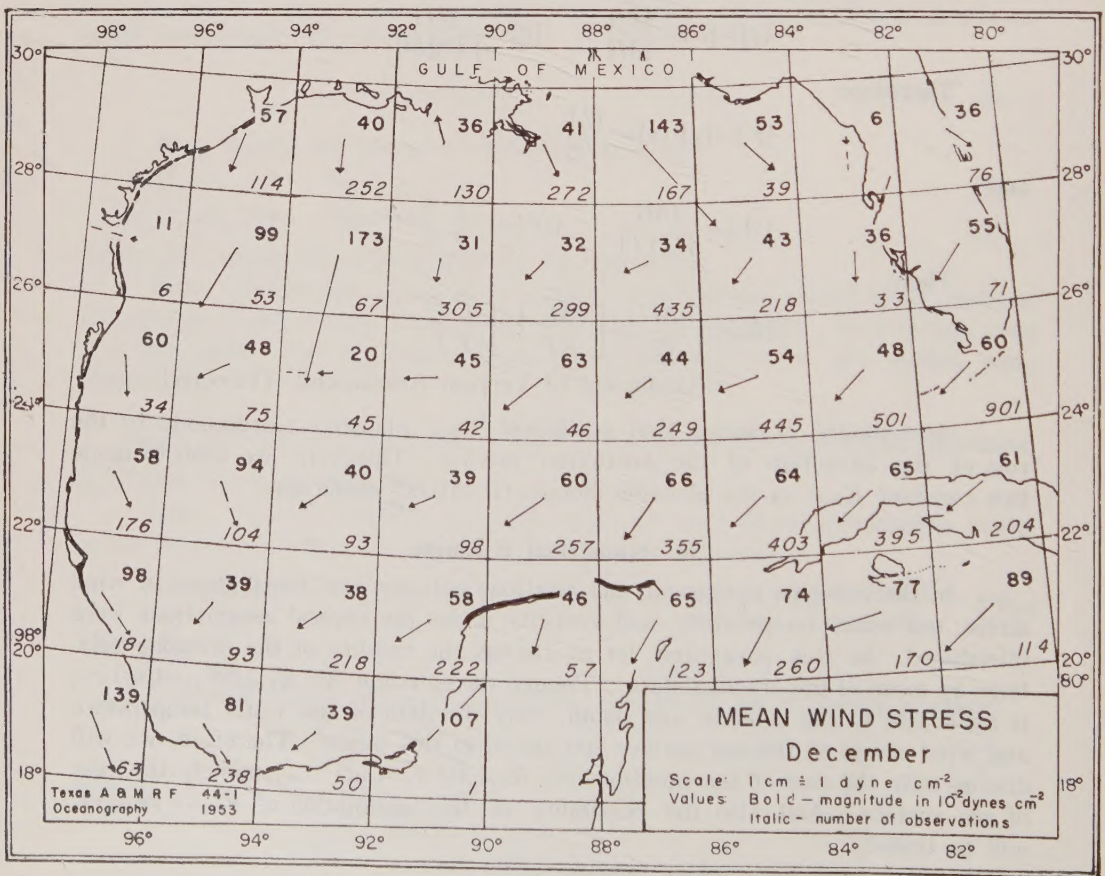
GULF OF MEXICO
MEAN WIND STRESS
December
Scale: 1 cm ≒ 1 dyne cm⁻²
Values: Bold – magnitude in 10⁻²dynes cm⁻²
Italic – number of observations
Texas A & M R F 44-1
Oceanography 1953

Fig. 1. Mean wind stress in the Gulf of Mexico (December)

stress in December over the Gulf of Mexico is shown by Fig. 1. These values in Fig. 1 were computed by Guy A. Franceschini, using the usual method.

The basic equation used is $\tau=\rho\mu^2V^2$, where τ is the wind stress (dyne/cm^2), ρ is the air density (gram/cm^3) at sea level, V is the wind speed (cm/sec) at a given height (10 meters) and μ is the resistance coefficient (dimensionless).

The resistance coefficient was assigned as follows.

$\mu_1{}^3=0.8\times10^{-3}$ (Hydrodynamically smooth surface).

$\mu_2{}^2=2.6\times10^{-3}$ (Hydrodynamically rough surface).

And he took the critical wind speeds marking the transition from the smooth surface to the rough surface as 670 cm/sec.

The monthly mean and the annual wind stress for each 2° quardangle (latitude and longitude) is presented by a vector originating at the center of the quardangle and drawn to a scale of approximately 1 cm per unit stress.

Now, take the rectangular axes Oxy on the sea surface, such that Ox and Oy towards the east and the north respectively. And let $\tau_x(o)$ and $\tau_y(o)$ denote the x and y components of wind stress vector respestively.

The each values of $\tau_x(o)$ and $\tau_y(o)$ were obtained by resolving the wind strees vector of Fig. 1 into x and y component at each stations.

These values $\tau_x(o)$ and $\tau_y(o)$ are shown by Table 1.

Table 1

The values of $\tau_x(o)$ and τ (o). (dyne/cm^2)

Lat.	Long.	97°	95°	93°	91°	89°	87°	85°	83°	81°
29°	x		− 0.18	− 0.04	− 0.10	+ 0.20	+ 0.90	+ 0.37	0.00	+ 0.30
	y		− 0.53	− 0.45	+ 0.37	− 0.43	− 1.05	− 0.38	− 0.10	− 0.26
27°	x	− 0.10	− 0.35	− 0.31	− 0.05	− 0.25	− 0.33	− 0.25	0.00	− 0.35
	y	0.00	− 0.83	− 1.71	− 0.31	− 0.25	− 0.15	− 0.33	− 0.40	− 0.47
25°	x	+ 0.08	− 0.40	− 0.20	− 0.43	− 0.53	− 0.34	− 0.52	− 0.40	− 0.43
	y	− 0.55	− 0.23	0.00	− 0.05	− 0.42	− 0.25	− 0.18	− 0.38	− 0.36
23°	x	+ 0.25	+ 0.25	− 0.35	− 0.34	− 0.52	− 0.40	− 0.45	− 0.45	− 0.50
	y	− 0.45	− 0.55	− 0.27	− 0.15	− 0.37	− 0.55	− 0.40	− 0.38	− 0.35
21°	x	+ 0.44	− 0.23	− 0.30	− 0.45	− 0.15	− 0.32	− 0.35	− 0.73	− 0.67
	y	− 0.50	− 0.30	− 0.22	− 0.32	− 0.45	− 0.55	− 0.62	− 0.17	− 0.55
19°	x	+ 0.40	+ 0.40	− 0.31	+ 0.72					
	y	− 0.75	− 0.50	− 0.25	+ 0.75					

Therefore, the value of Curl $\tau_{(o)}$ at each stations were computed by using the numerical differentiaing method.

The respective values of the center of the square point pattern were computed by interportation method. These values are shown in 10^{-2} (dyne cm^2/mile.) unit.

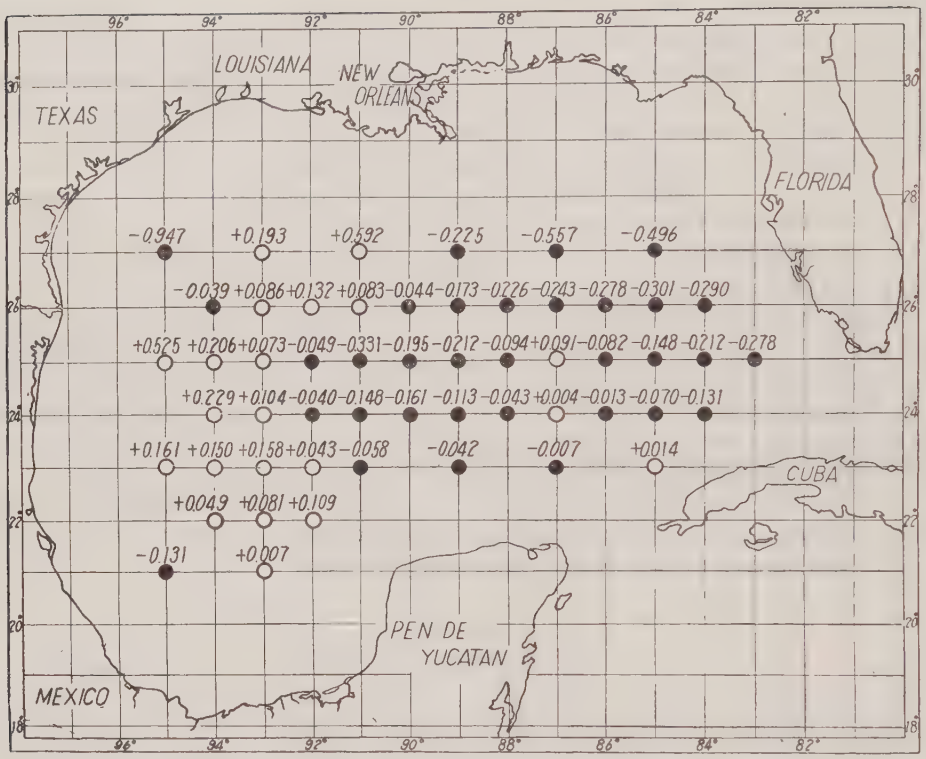

Fig. 2. Distribution of the value of $Curl\ \tau_{(0)}$. The magnitude of the value is in $\times 10^{-2}$ (dyne/cm^2/mile)

○ ···· + sign ● ····· — sign

In the Fig. 2, white circles mean the positive value of Curl $\tau_{(0)}$ and black circles the negative value of Curl $\tau_{(0)}$. The former means the sinking and the latter the upwelling.

Computation of $\Delta^2\theta_{(0)}$

Fig. 3 shows the mean distributions of sea surface temperature (°F) in the Gulf of Mexico in December. The tabulation of isothermal lines in this figure were presented from the data (1), and we determined the values of each stations by means of interpolations method, referring those isothermal lines.

These values in Fig. 4 are indicated by Celsius expression. The numerical computation of $\Delta^2\theta_{(0)}$ was carried by the following formula.

$$\nabla^2\theta_{(0)}=\frac{\partial^2\theta_{(0)}}{\partial x^2}+\frac{\partial^2\theta_{(0)}}{\partial y^2}\approx\frac{1}{\Delta x^2}\left\{\theta_{i+1,j}(o)-2\theta_{i,j}(o)+\theta_{i-1,j}(o)\right\}+\frac{1}{\Delta y^2}\left\{\theta_{i,j+1}(o)-2\theta_{i,j}(o)+\theta_{i,j-1}(o)\right\},$$

where, $\Delta x=50$ mile, $\Delta y=60$ mile.

and $\Delta y=\frac{6}{5}\Delta x$, then we have

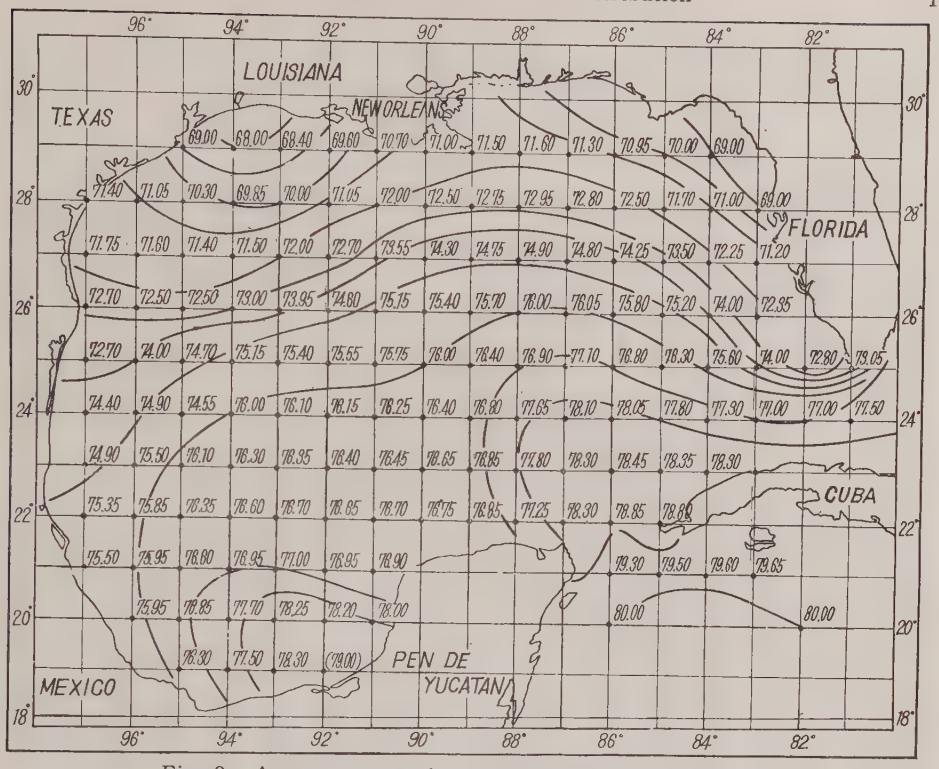

Fig. 3. Average sea surface temperature (°F) December.

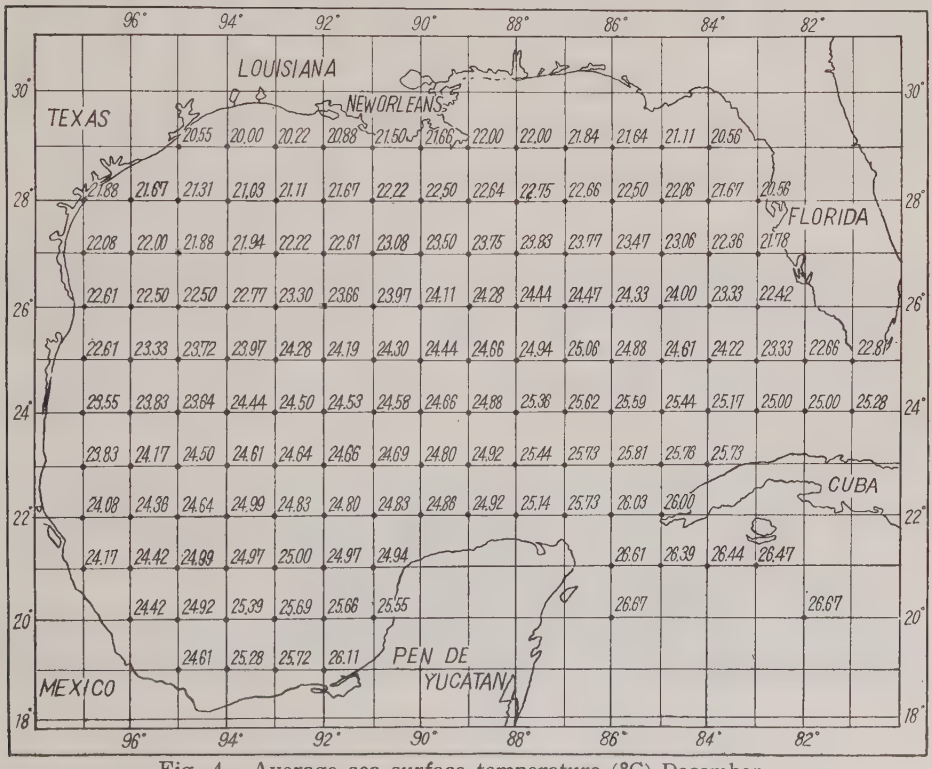

Fig. 4. Average sea surface temperature (°C) December.

$$\nabla^2\theta_{(o)} = \frac{1}{2500}\left\{[\theta_{i+1,j}(o) - 2\theta_{ij}(o) + \theta_{i-1,j}(o)] + \frac{5}{6}[\theta_{i,j+1}(o) - 2\theta_{i,j}(o) + \theta_{i,j-1}(o)]\right\} .$$

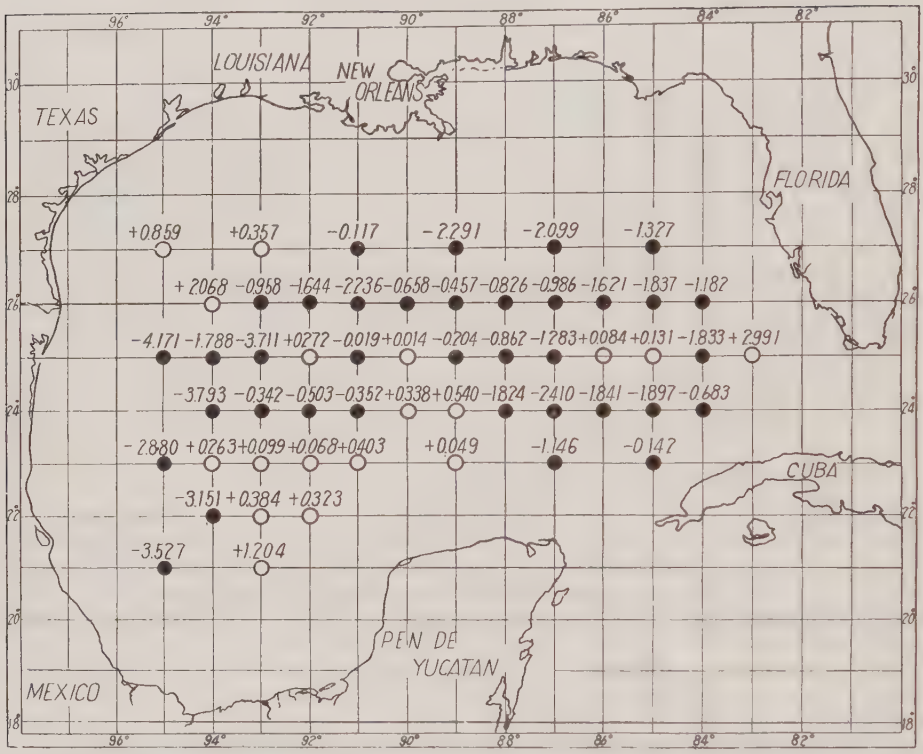

Fig. 5. Distribution of the value of $\nabla^2\theta_{(o)}$. The magnitude of the value is in $\times 10^{-4}$ (1/mile^2). ○ ···· + ● ···· −

The distributions of the values of $\Delta^2\theta_{(o)}$ are shown in Fig. 5. In this map white circles mean $\Delta^2\theta_{(o)} > o$, and black circles $\Delta^2\theta_{(o)} < o$. The former means the sinking and the latter the upwelling. The magnitude of values is in 10^{-4}/mile^2.

The Fig. 6 shows the distribution of the values of Curl $\tau_{(o)}$ and $\Delta^2\theta_{(o)}$ which were obtained by the extraction of the corresponding points having the same sign in Fig. 2 and Fig. 5.

White circles show Curl $\tau_{(o)} > o$ and $\Delta^2\theta_{(o)} > o$, therefore they mean sinking, and black circles Curl $\tau_{(o)} < o$ and $\Delta^2\theta_{(o)} < o$, therefore they mean upwelling. The upper numbers above each circles indicate the values of $\Delta^2\theta_{(o)}$ at each stations, and the lower numbers the values of Curl $\tau_{(o)}$.

In Fig. 6, the upwelling regions are mainly situated in latitude 20°～30°N, longitude 92°～94°W. The sinking region are situated in latitude 24°～27°N, longitude 84°～91°W. The later region corresponds to that of the prevaling

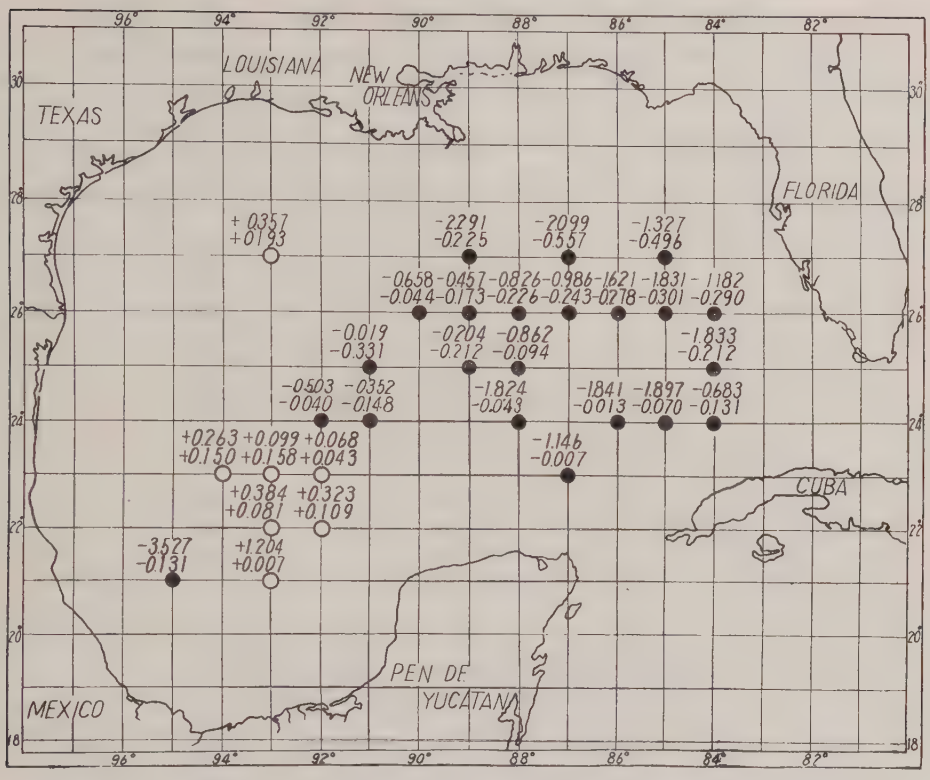

Fig. 6. Distribution of $\nabla^2\theta_{(k)}$ and $Curl\ \tau_{(0)}$. Point pattern shows sign of $\nabla^2\theta_{(0)}$ and Curl $_{(0)}$ as follows

○ ····· $\begin{cases} \nabla^2\theta_{(0)}>0 \\ Curl\ \tau_{(0)}>0 \end{cases}$ ● $\begin{cases} \nabla^2\theta_{(0)}<0 \\ Curl\ \tau_{(0)}<0 \end{cases}$

The upper-number shows the value of $\nabla^2\theta_{(0)}$, and the lower shows the value of $Curl\ \tau_{(0)}$. The magnitude of $\nabla^2\theta_{(0)}$ is in $\times 10^{-4}$ (1/mile^2) and the magnitude of $Curl\ \tau_{(0)}$ is in $\times 10^{-2}$ (dyne/cm^2/mile).

current. And in this region the distributions of Fig. 2 and Fig. 5 coinside well with each other. But, unfortunately in the region of the upper corner of left hand side in our mup, we can not find the necessary coinsident points in Fig. 2 and Fig. 5. We will discuss about this point in the later paragraph.

Next, we will compute the values of $K_{p(0)}$ at each stations by use of the equation (36),

$$K_{p(0)}=\frac{Curl\ \tau_{(0)}}{\nabla^2\theta_{(0)}}\ .$$

These values are shown in Fig. 7 expressing the distribution of $K_{p(0)}$ in C.G.S. unit, where the dimension of $K_{p(0)}$ is

$$[K_{p(0)}]=\left[\frac{M}{T^2}\right]\left(=\left[\frac{M}{LT}\right]\cdot\left[\frac{L}{T}\right]\right)\quad (\text{gram/sec}^2).$$

Determination of coefficient γ.

In the paragraph 2, it was assumed that the specific volume of sea water

Table 2
An average temperature-salinity correlation for the Gulf of Mexico proper.
(After Parr)

Average sea temperature	Average salinity	Weighted Average depth
24.74 (C°)	36.19 (‰)	5 (m)
23.06	36.06	15
21.03	36.14	58
19.25	36.28	94
17.09	36.22	125
14.85	35.95	192
13.00	35.68	237
10.89	35.35	321
9.60	35.16	380
8.57	35.04	432
7.42	34.93	562
6.39	34.88	647

Table 3
An average temperature-salinity in the Gulf of Mexico.
(Obtained from the interporation method.)

Depth (*m*)	Temperature (C°)	Salinity. (‰)	Chrolinity. (‰)
0			
5	24.74	36.19	20.03
10	23.73	36.09	19.98
15	23.06	36.06	19.96
20	22.63	36.05	19.95
25	22.25	36.05	19.95
30	21.95	36.06	19.96
50	21.22	36.11	19.99
75	20.35	36.22	20.03
100	18.88	36.28	20.08
150	16.50	36.15	20.01
200	14.45	35.89	19.87
250	12.60	35.50	19.65
300	11.32	35.38	19.59
400	9.18	35.11	19.44
500	7.93	34.98	19.37
600	7.10	34.88	19.31

α is proportional to the sea water temperature $\theta_{(z)}$, namely $\alpha=\gamma\theta+c$, where γ means the proportional coefficient.

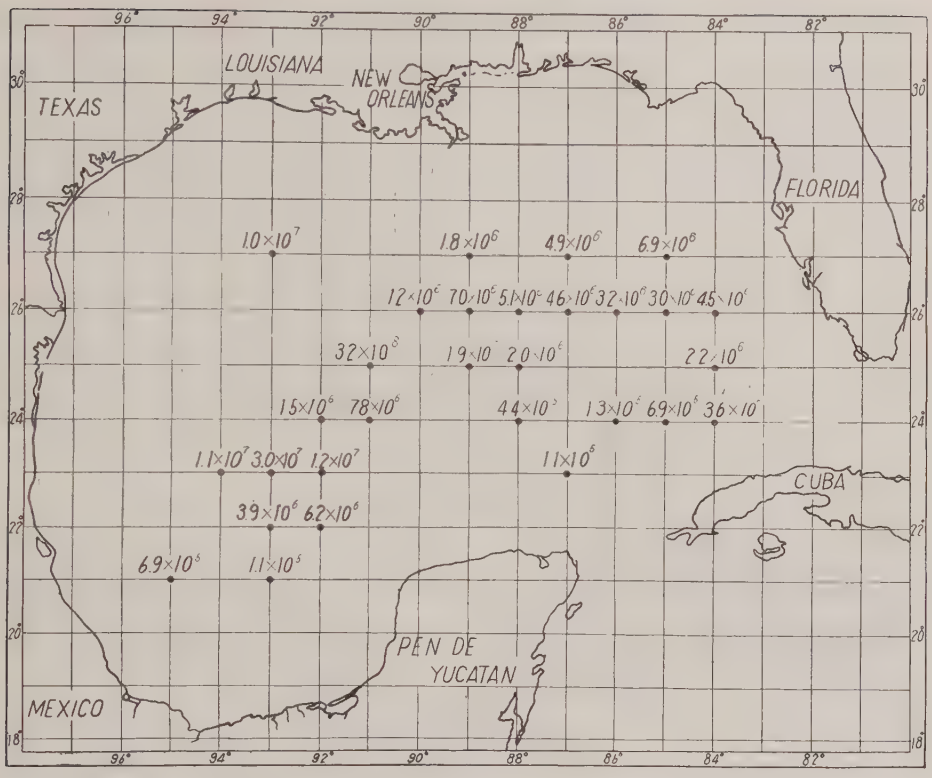

Fig. 7. Distribution of the value of $K_p(o)$ in C.G. S unit (gram/sec²)

The mean values of salinity and temperature over the Gulf of Mexico from data (2) are given in Table 2.* (These mean values were given by Parr in 1935).

And the result from Table 2 is plotted in Fig. 8.

The interporated respective values are shown in Table 3.

Using the value of Table 3, we computed the values of $\alpha_{s,\theta,z}$. And the relation-curve of $\alpha_{s,\theta,z}$ and θ_z is shown in Fig. 9. Glancing at this curve, its curvature is small. Accordingly, it is not unreasonable to replace approximately the given relation curve by a straight line. Therefore, we can put the following relation,

$$\alpha_{s,\theta,z}=\gamma\theta+c$$

Then, we have

$$\gamma\approx\frac{\Delta\alpha_{s,\theta,z}}{\Delta\theta}$$

From this results, we get the following mean value of γ.

The computation of γ was carried out by the above formula. (Refer Table 4).

* These values corresponds to those in the season of February～April. Though the season treated by us was December, as an example of cold season was adopted this case, because of the absence of data in December.

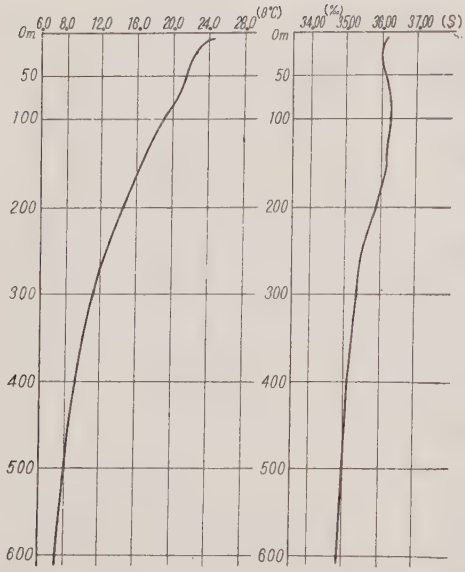

Fig. 8 (1) Ploted out from Table-2. Horizontal axis shows the value of sea water temperature, and vertical axis the depth.

Fig. 8 (2) Horizontal axis shows the value of salinity, and vertical axis the depth.

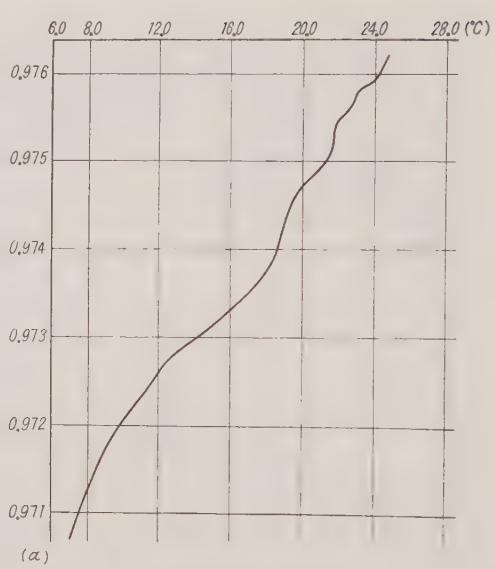

Fig. 9. The relation between $\theta_{(z)}$ and $\alpha_{s,\theta,z}$. Horizontal axis shows the value of temperature, and vertical axis the value of specific volume.

Table 4

The values of $\alpha_{s,\theta,z}$ and γ.

z	$\theta_{(z)}$	$\Delta\theta_{(z)}$	$\alpha_{s,\theta,z}$	$\Delta\alpha_{s,\theta,z}$	$\gamma=-\frac{\Delta\alpha_{s,\theta,z}}{\Delta\theta_{(z)}}$
0					
5	24.74		0.97622		
10	23.73	1.01	588	0.00034	3.4×10^{-4}
15	23.06	0.67	581	07	1.0×10^{-4}
20	22.63	0.43	563	18	4.2×10^{-4}
25	22.25	0.38	554	09	2.3×10^{-4}
30	21.95	0.30	543	11	3.7×10^{-4}
50	21.22	0.73	497	46	6.3×10^{-4}
75	20.35	0.87	482	15	1.7×10^{-4}
100	18.88	1.47	420	62	4.2×10^{-4}
150	16.50	2.38	352	68	2.8×10^{-4}
200	14.45	2.05	306	46	2.2×10^{-4}
250	12.60	1.85	276	30	1.6×10^{-4}
300	11.32	1.28	241	35	2.7×10^{-4}
400	9.18	2.14	180	61	2.8×10^{-4}
500	7.93	1.25	124	56	4.5×10^{-4}
600	7.10	0.83	074	50	6.0×10^{-4}
				(Mean value)	3.3×10^{-4}

From this results, we get following mean value of γ,

$$\gamma=3.3\times10^{-4}\ (\mathrm{cm^3\ gram^{-1}})$$

Accordingly, $\gamma'=\eta\cdot\gamma=3.3\times10^{-1}\ (\mathrm{cm\ sec^{-2}})$

The condition of $\alpha=\gamma\theta+C$ plays a important role in our treatment. If this approximate condition will not be established in our sea region considered, our equation can not be deduced.

Value of $A_z(o)$.

In the equation (36), we put $K_{p(o)}=\dfrac{A_z(o)\gamma'}{2w\sin\varphi}$.

In this case, we will put $\varphi=25.°$ $\omega=7.292\times10^{-2}\ (\mathrm{sec^{-1}})$, $\gamma'=3.3\times10^{-1}\ (\mathrm{cm\ sec^{-2}})$. Then it follows that,

$$K_{p(o)}=\frac{3.3\times10^{-1}\,A_z(o)}{2\times7.292\times10^{-5}\sin25°}\,.$$

Accordingly, $A_z(o)=1.86\times10^{-4}\times K_p(o)$.

Therefore, we can get the values of $A_z(o)$ at each stations by means of the values of K_p already given in Fig. 7. The results are shown in Fig. 10.

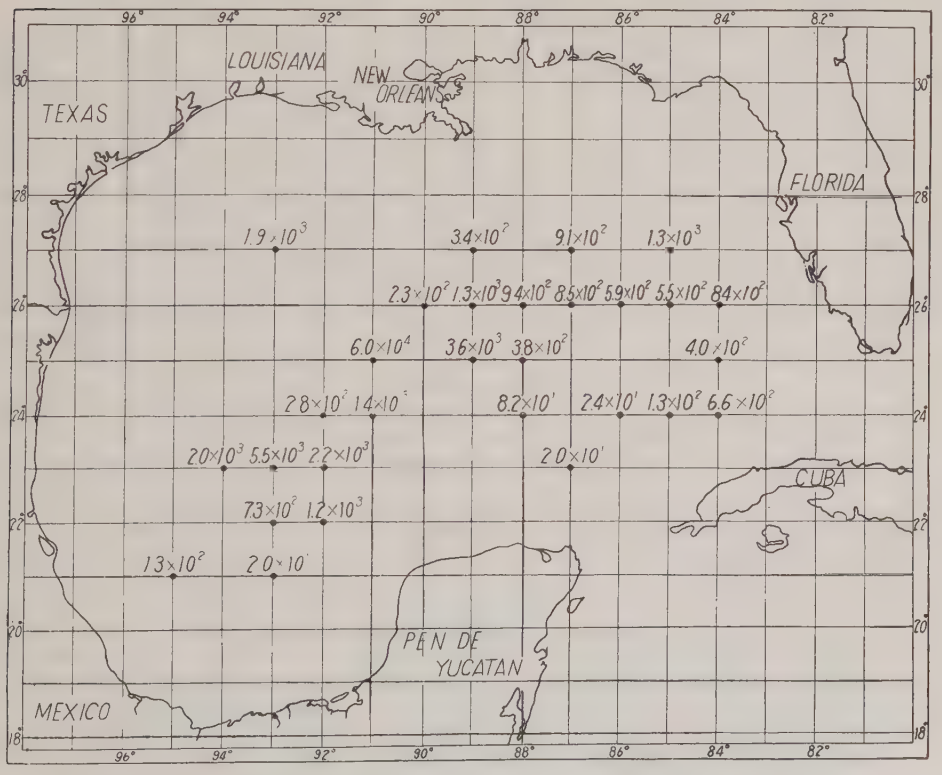

Fig. 10. Distribution of the value of $A_z(o)$ in C.G.S unit. (gram/cm sec).

4. Discussion and conclusion.

Now, let us consider the distribution of values of $K_{p(o)}$ and $A_z(o)$. (Refer Fig. 7 and Fig. 10). As seen from Fig. 7, the values of $K_p(o)$ are distributed between 10^5 and 10^7 (gram/sec^2) in order of magnitude. The values in the regions between latitude 23°~25°N, and longitude 89°~94°W. are relatively larger (10^7 and 10^8 C.G.S.).

In the northern and eastern parts of Gulf, the values are 10^6 (C.G.S.) in order of magnitude and the southern part 10^5 (C.G.S.).

The distribution of $A_z(o)$ is proportional to that of $K_p(o)$,

where $[K_p(o)]$=[the Coefficient of Austaushi]×[velocity].

Therefore, it is possible that in the regions of stronger horizontal current velocity, the values of $K_{p(o)}$ are also large. In fact, this tendency is found in Fig. 7 and Fig. 11.

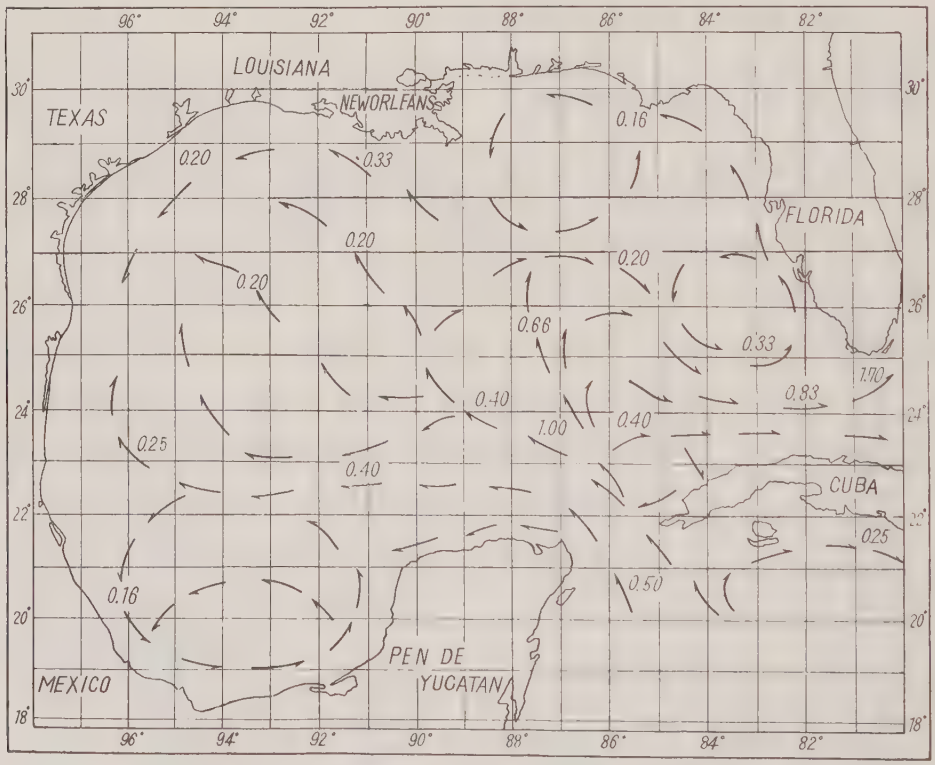

Fig. 11. Average sea surface currents (Mile per hour).

And the values of $A_z(o)$ show the magnitude between 10^1 and 10^4 (C.G.S.) in order of magnitude, and the varaity of these values is as follows.

Order of $A_z(o)$ (gram cm^{-1} sec^{-1})	10^4	10^3	10^2	10^1
Numbers of station	1	9	14	4

Therefore, omitting the order of magnitude 10^4 (C.G.S.), we get the following mean values of $A_z(o)$:

$$\overline{A_z(o)} = 9.22 \times 10^2.$$

It seems that this value is reasonable in the case of our sea region, referring from the values which have been already get in the various sea regions (in Pacific and Atlantic oceans). Therefore, considering from the adequteness of the value of $A_z(o)$, we will be able to recognize the validity of the equation (36).

Accordingly, if we can get the mean values of $A_z(o)$, γ (from the previous observation) and the distribution of wind stress, it will be possible to obtain the mean distribution of surface water temperature by solving the differential equation (36) with the boundary conditions concerning the water temperature. But, this formula is not applicable to the case of the wide sea region, because in our treatment it was assumed that the term of Coriolis parameter l $(=2\omega \sin \varphi)$ is constant. Therefore, when we intend to apply this formula to the case of wide sea region, it will be noted that the sea region considered should be divided into the suitable zonal regions and then the formula (36) is able to use to the divided respective regions.

As stated in the previous paragraph, in the corner sea region in the upper left hand side of our map (refer Fig. 6), our equation (36) was not satisfied.

It is supposed that this fact is due to the following reason. In our original equations of motion, the horizontal frictional and non-linear terms were neglected. However, in the intermediate sea region between the weak current zone and the strong current zone, these terms, especially the term of horizontal frictional resistance cannot be neglected.

Therefore, in order to perform the more correct treatment, these terms (especially, the horizontal frictional term) must be introduced in our equations of motion. And also we can not neglect the effect of ocean current inflowing from the channel between PEN DE Yukatan and Cuba Island. The treatment by the introduction of this conception will be discussed in the future. Finally, it should be noticed that the condition $\alpha = \gamma\theta + c$ plays a important role in our treatment.

Acknowledgement

Closing this paper, the author wishes to express his hearty thanks to Mr. Tadashi Yoshioka for his carefull numerical computation and assistance in the preparation of this paper.

The research reported in this paper has been sponsored by the Ministry of Education.

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Journal of the Tokyo University of Fisheries, Vol. 46, Nos. 1–2, 1960

MARINE ALGAE FROM LAKE SAROMA, HOKKAIDO

Kozo Iwamoto

(With Plates 1–15.)
(Received Feb. 5, 1960)

Since 1944 the writer has tried seven collecting journeys in Lake Saroma, mainly in summer. The most abundant algal flora was found at Sakaeura located at north-easten end of the lake.

The present paper includes 62 species in all which contain 11 Chlorophyceae, 21 Phaeophyceae and 30 Rhodophyceae, among which *Myriactula saromaensis*, *Punctaria kinoshitae*, *Punct. tenuis* and *Punct. conglomerata* have been investigated with Prof. Y. Yamada and are described as new species, and *Ascocyclus orbicularis*, *Dictyosiphon corymbosum*, *Peyssonelia dubyi* and *Chondria polyrhiza* are described as new to Japanese algal flora.

The present study was carried under the guidance of Prof. Y. Yamada, and the present paper has been left for about 10 years by the reason of writer's long illness. Now, for the publishing of the present paper the writer is much indebted to a great deal of kindness offered by many people. The writer to offer his best thanks to his teacher Prof. Y. Yamada of the Department of Botany, Faculty of Science, Hokkaido University, who gave the most valuable suggestions and kind guidance, and to Dr. T. Kinoshita of the Hokkaido Regional Fisheries Research Laboratory, who gave many facilities for his collecting journeys. Thanks are also due to all gentlmen, who helped him during his collecing journeys, especially to the late Mr. K.Kajiwara at Sakae-ura, who kindly helped him from the beginning to the end of his collection, to Mr. K. Shin-ya, the headman of Tokoro Town, and to Mr. Y. Kikuchi of the Yubetsu Fisheries Association, who gave many facilities to the writer during his journeys. And to Prof. S. Ueda and Mr. A. Miura of the Department of Botany, the Tokyo University of Fisheries, who gave many facilities and helps, he wishes to offer his thanks.

Some of the expense of the journey was defrayed by the Hokkaido Fisheries Scientific Institution, to the director of which in those days the writer wishes to extend his sincere thanks.

Remarks on Lake Saroma

Reference literature: Saroma-ko, the Suisan Chosa Hokoku, Vol. 34, 1934 (in Japanese), the Hokkaido Fisheries Scientific Institution, Yoichi, Hokkaido.

Lake Saroma located near Abashiri City in the north-eastern coast of Hokkaido is a kind of lagoon, which is partitioned from Okhotsk Sea by two sand bars, having the width of 150 meters, being about 150 square kilometers in area and about 90 kilometers in circumferance, and forms a quadrilateral elongated to east and west. The lake is connected with Okhotsk Sea by a opening being about 300 meters in width, so the water in the lake is intermingled with that of the open sea by the tide. The opening was artificially made at the rather

western portion of the sand bar in 1924, and till that time a natural opening had been located at Tobutsu, the north-eastern end of the lake.

The greater part of the coast is sandy, but the rocky boards are exposed at several projections in the southern coast, that is, Horoiwa, Tokotan, Maruyama and especially at the south-eastern coast of Baro.

The main rivers pouring into the lake are the Baro, the Shibushi, the Kerochi, the Tokotan, the Saroma-betsu and the Raitokoro. River Raitokoro is almost stagnant and makes a swamp at the outside of the north-eastern coast.

Fig. 1. Lake Saroma

The lake is rather shallow, even at the deepest portion 19 meters deep, and very quiet in all seasons. The southern projecting coasts are rather steep, but in the other coasts the shoals generally extend to the offing. And at Sakae-ura penetrating into the north-eastern coast the great quantities of oyster shells

and the decayed leaves of *Zostera* are accumulated here and there. In the mouth of this penetrating waters the depth of water becomes only 10–20 cm. when the tide is low, and the tidal current is rapid, and the bottom is composed of sand, pebbles and shells.

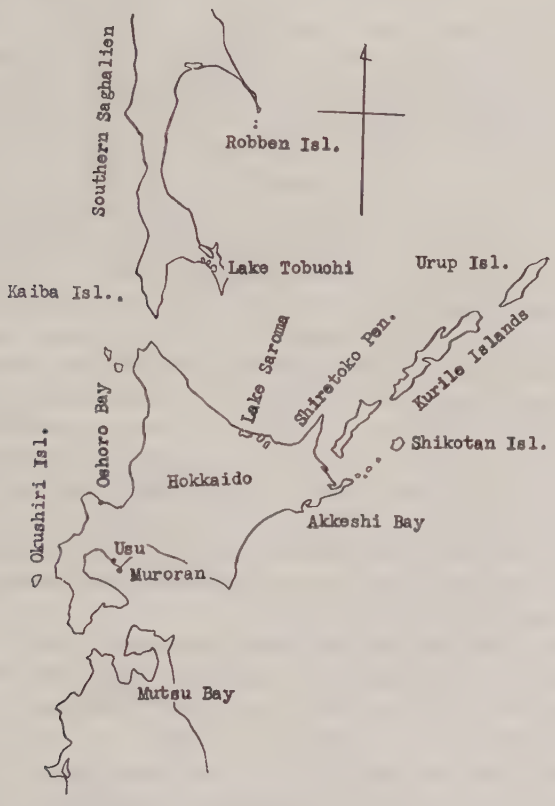

Fig. 2. Hokkaido and its vicinity

There is a difference less than 60 cm. between the depths of water at low and high tides in this lagoon.

In summer the temperature of water attains about 20°C near the surface, especially at Baro to Teinei and at Sakae-ura the temperature of water attains higher degree because of the water is rather stagnant. In winter the water begins to freeze at the beginning of December and is frozen over the whole surface, at that time the temperature of water is –0.5°C — –1.5°C, and there is only a small difference between the temperatures of upper and lower waters.

The chlorine quantity of water is about 18 gr. per liter, that is, in the western part of the lake it is about 18 gr., in the central it does not attain 18 gr., and at the vicinity of Tobutsu and the mouth of River Raitokoro, it is less than 17 gr.

As to the flora of this lake, *Zostera marina* grows along the whole coast except southern rocky coasts, but especially thick along the eastern coast, from

Sakae-ura to Kawaguchi, and north-eastern coast, from Baro to the mouth of the lake including Teinei, Naka-ban-ya and Sanri-ban-ya. From Baro to Teinei *Zostera marina* grows thick to far offing. In addition to *Z. marina* a small quantity of *Z. nana* is dispersely found also at Sakae-ura and along the northern coast.

The algae have been found abundantly at Sakae-ura and at Baro. At Sakae-ura many kinds of algae grow on the shells, on the pebbles, on the leaves of *Zostera* and on various algae. Both *Dictyosiphon foeniculaceus* and *Dasya sessilis* are impressive seaweeds at Sakae-ura, the former grows well on *Scytosiphon* in June, and the latter, however, grows on the shells washed by the tidal current, and is fructiferous in August. A few dimunitive and sterile fronds of *Dasya* are found also on the rocks at Baro in August. Many fronds of fructifying *Chrysymenia wrightii* have been collected at Sakae-ura from the depth of 1.5–2 meters in summer, which have grown on living shells of oyster. It is interesting that *Antithamnion sparsum* and *Ant. corticatum* which had been described by Prof. J. TOKIDA from Lake Tobuchi, the lagoon in Saghalien, have been collected also on the same oyster shell as mentiond above. *Chondrus pinnulatus* has been found also at the same habitat, however, these fronds are thinner and less ramified than those growing on the rocks at Baro and Tokotan located on the southern projecting rocky coasts. In addition to this species, *Gymnogongrus japonicus*, *Laurencia nipponica*, *Rhodomela larix* and *Corallina pilulifera* have been found also at these rocky coast A quantity of dimunitive fronds of *Gloiopeltis furcata* has been found at Tokotan only. *Asterocytis ornata*, *Goniotrichum alsidii* and *Erythritrichia carnea* have been found frequently on the tetrasporic plants of *Polysiphonia japonica* collected at Baro.

It should be mentioned specially that the great quantity of several species of *Punctaria* appears in the coast of Naka-ban-ya to the mouth of lake in July. At the same time *Rhododermis georgii* grows on the edges of the decayed leaf of *Zostera* on the coast.

On the other coast the algal flora is very poor, that is, along the sandy coast *Enteromorpha*, *Ulva* and *Gracilaria* have been found. *Gracilaria* has been found also at rocky coasts. It is said that a certain species of *Laminaria* grows in the mouth to central part of the lake, but the writer had not any chance to collect this *Laminaria*.

At last the writer wishes to inform that *Ahnfeltia plicata*, from which agar is made, had been transplanted from Isl. Kunashiri, Kurile Islands, by the Hokkaido Fisheries Scientific Institution, it seems, however, that its propagation is not so favourable. On the other hand the cultivation of *Porphyra yezoensis*, which is transported in the state of germ on the culture net from Usu located near Muroran City, is carried by the Institution mentioned above, and this cultivation shows a good result.

CHLOROPHYCEAE

Ulvaceae

1. *Enteromorpha intestinalis* (L.) LINK

1820, p. 5; J. AGARDH, 1883, p. 131; BOERGESEN, 1902, p. 489; COLLINS, 1903,

p. 23; 1909, p. 204; Collins and Hervey, 1917, p. 34; Setchell and Gardner, 1920, p. 252; Nagai, 1940, p. 12; Dawson, 1944, p. 203; Bliding, 1948, p. 123; Kylin, 1949, p. 22, fig. 13; Tokida, 1954, p. 54.

Jap. name: Yore-awonori.

Hab.: Floating in the intertwined masses in summer.

2 *Enteromorpha compressa* (L.) Greville

1830, p. 130, pl. 18; Harvey, 1851, pl. 335; Kuetzing, 1856, pl. 38; J. Agardh, 1883, p. 137; Collins, 1903, p. 25; 1909, p. 21; Setchell and Gardner, 1920, p. 251; Dawson, 1944, p. 203; Bliding, 1948, p. 126; Kylin, 1949, p. 22, figs. 14, 15.

Jap. name: Hira-awonori.

Hab.: Sakae-ura and Baro. Growing on shells, stones and rocks in the lower tidal belt in summer.

3. *Enteromorpha plumosa* Kuetzing

1843, p. 300, pl. 20, fig. 1; Hauck, 1885, p. 430, fig. 189; Collins, 1909, p. 198; Collins and Hervey, 1917, p. 33; Setchell and Gardner, 1920, p. 259; Tokida, 1954, p. 53.

Jap. name: Watage-awonori.

Had: Sakae-ura. Growing on shells and pebbles in the lower tidal belt washed by the tidal current. July.

The difference between *E. plumosa* and *E. crinita* is not so clear. According to the investigations of Setchell and Gardner (1920) and Collins (1909), in *E. plumosa* the chromatophores do not fill the cells, but those of *E. crinita* fill the cells. Collins has also described that the latter occasionally has the short monosiphonous branches, and on the contrary the former has the considerably extended ones. Judging from these informations it seems to the writer that whether the chromatophores fill the cells or not is a more important characteristic than the length of the monosiphonous branches so the writer identified the present specimens with *E. plumosa*.

4. *Enteromorpha crinita* (Roth) J Agardh

1883, p. 144; Collins, 1903, p. 26; 1909, p. 199; Setchell and Gardner, 1920, p. 258; Taylor, 1945, p. 39.

Jap. name;

Hab.: Sakae-ura and Baro. Growing on shells and pebbles in the lower tidal belt. July.

The present specimens have the considerably longer monosiphonous branches than those of Saroma's *E. plumosa*, besides in the former the cells consisting the monosiphonous ones often project here and there.

5. *Enteromorpha linza* (L.) J. Agardh

1883, p. 134; Boergesen, 1902, p. 489; Collins, 1903, p. 23; 1909, p. 206; Okamura, 1915, p. 163, pl. 138; Kylin, 1949, p. 19, fig. 18; Tokida, 1954, p. 55.

Ulva linza L., Setchell and Gardner, 1920, p. 262

Jap. name: Usuba-awonori.

Hab: Sakae-ura. Growing on shells and pebbles washed by the tidal current. In spring.

6. *Ulva pertusa* KJELLMAN
1897, p. 4; OKAMURA, 1921, p. 79, pl. 67; TOKIDA, 1954, p. 57.
Jap. name: Awosa
Hab.: Common along the coast.

7. *Monostroma zostericola* TILDEN
YENDO, 1917, p. 184; SETCHELL and GARDNER, 1920, p. 238; YAMADA, 1935, p. 9; KAWABATA, 1936, p. 199; NAGAI, 1940, p. 20, pl. 1, figs. 27, 28; TOKIDA, 1954, p. 59, pl. VI, figs. 1–3.
Monostroma leptodermum COLLINS, 1909, p. 213.
Jap. name: Motsuki-hitoe.
Hab: Sakae-ura. Epiphytic on leaves of *Zostera* in July.

Cladophoraceae

8. *Chaetomorpha crassa* (J. AGARDH) KUETZING
1853, pl. 59; HAUCK, 1885, p. 439; OKAMURA, 1931, p. 97; YAMADA, 1934, p. 42.
Jap. name: Hoso-jyuzumo.
Hab.: Entangled to *Sargassum miyabei*. Whole year.
The summer plants are softer than the winter ones, and the latter are of conspicuously stiff texture. In respect to the colour the latter fades remarkably and become pellucid.

9. *Cladophora stimpsonii* HARVEY
COLLINS, 1909, p. 338; SETCHELL and GARDNER, 1920, p. 219; YAMADA, 1928; p. 501, fig. 4; TOKIDA, 1954, p. 43, pl. II, figs. 1–4.
Jap. name: Kinu-shiogusa.
Hab.: Sakae-ura and Baro. Growing in tangled masses among *Sargassum* forest.

10. *Spongomorpha saxatilis* (RUPRECHT) COLLINS
1909, p. 360; SETCHELL and GARDNER, 1920, p. 226; KAWABATA, 1940, p. 34, figs. 6, 7; SAKAI, 1954, p. 76, fig. 5.
Cladophora saxatilis (RUPR.) DE TONI, 1889, p. 311; SETCHELL and GARDNER, 1903, p. 223.
Jap. name: Togenashi-motsuregusa.
Hab.: Sakae-ura. Growing on shells washed by the tidal current in the lower tidal belt. In July.

Bryopsidaceae

11. *Bryopsis plumosa* (HUDSON) C. AGARDH
HAUCK, 1885, p. 471, fig. 208; BOERGESEN, 1907, p. 147; 1911, p. 48, fig. 7; COLLINS, 1909, p. 403; SETCHELL and GARDNER, 1920, p. 14, figs. 1, 2.
Jap. name: Hane-mo.
Hab.: Sakae-ura. Growing on oyster shells in the depth of 2 m. and about

5 mm. tall individuals growing on *Chondrus pinnulatus* are collected from the same habitat at the middle of June.

Judging from the arrangement of the pinnules, it seems to the writer that the present plants are far away from the typical form of this species, however, in the present one the arrangement of pinnules is not so irregular as those of *B. hypnoides*, so the writer has identified them with *B. plumosa* in some hesitation.

PHAEOPHYCEAE

Ralfsiaceae

12. *Ralfsia verrucosa* (Areschoug) J. Agardh
1848, p. 62; Kuetzing, 1859, pl. 77, fig. 2; Reinke, 1889a, p. 48; 1889b, pls. 5, 6, figs. 1–13; Yendo, 1918, p. 65; Kylin, 1947, p. 44; Tokida, 1954, p. 82.
Jap. name: Iso-iwatake.
Hab.: Sakae-ura and Baro. Growing on pebbles and rocks in June

Myrionemataceae

13. *Ascocyclus orbicularis* (J. Agardh) Magnus
1874, p. 73; Kylin, 1917, p. 39; Boergesen, 1926, p. 63; Taylor, 1937, p. 158.
Myrionema orbiculare Hauck. 1885, p. 321.
Jap. name:
Hab.: Naka-ban-ya. Epiphytic with various small algae on leaves of *Zostera* in summer.

Elachistaceae

14. *Halothrix tortuosa* Takamatsu
Pl. 12, C, D.
1938, p. 184.
Jap. name:
Hab.: Naka-ban-ya. Epiphytic on leaves of *Zostera* in summer.

Of this species the writer has collected only once a few specimens growing on the leaves of *Zostera*. They seem to come very nearly to, or are probably the same as *H. tortuosa* Takamatsu which was described from the province of Rikuchu and Rikuzen. But there are some discrepancies between the original description and the present specimens. The cells of the assimilatory filaments reach about 60 μ in diameter and are 1–1,5–2 times as long as wide in the present ones, while Takamatsu reports them being 55 μ in the thickest portion of the assimilatory filaments and as long as the diameter. In unilocular sporangia there is also a difference: they are 75–94 × 25–36 μ in the present ones, considerably larger than those in the original description. The paraphyses in the Saroma specimens are consisted of slightly more cells than the Takamatsu's.

Sphacelariaceae

15. *Sphacelaria variabilis* SAUVAGEAU
1901, p. 160; YAMADA, 1928, p. 504; OKAMURA, 1936, p. 152; TAKAMATSU, 1938, p. 9; TOKIDA, 1954, p. 75.
Jap. name: Matazaki-kurogashira.
Hab.: Growing on the stem of *Sargassum miyabei*.

Dictyotaceae

16. *Dictyota spathulata* YAMADA
1928, p. 515.
Jap. name: Hera-amijigusa.
Hab.: Common in summer.

Leathesiaceae

17. *Leathesia difformis* (L.) ARESCHOUG
1847, p. 376; KUCKUCK, 1894, p. 248; OKAMURA, 1907, p. 80, pl. 18, figs. 1–14; SETCHELL and GARDNER, 1925, p. 511, pl. 40, fig. 52, pl. 43, figs. 65, 66; TAYLOR, 1937, p. 145, pl. 12, fig. 8; NAGAI, 1940, p. 43; KYLIN, 1947, p. 53; TOKIDA, 1954, p. 88.
Jap. name: Nebari-mo.
Hab.: Naka-ban-ya and Baro. Growing on rocks and on leaves of *Zostera* in summer.

18. *Myriactula sargassi* (YENDO) FELDMANN
Pl. 11.
1943, p. 224.
Myriactis sargassi YENDO, 1920, p. 3.
Gonodia sargassi (YENDO) SETCHELL and GARDNER, 1924, p. 722; TOKIDA, 1954, p. 85, pl. IX, fig. 3.
Jap. name: Gonokenori.
Hab.: On ramuli of *Sargassum miyabei* in summer.
The writer has not been able to see the YENDO's specimens, it is sure, however, that the Saroma specimens agree quite well with the original desciption of YENDO.

19. *Myriactula saromaensis* YAMADA and IWAMOTO spec. nov.
Pl. 3.
Frons epiphytica, fere hemisphaerica, 0.3–0.6 mm. diam. basi penetrali in hostem; filamentis assimilatoribus rectis, ca. 170 μ longis, 10–15 cellulis, cellulis inferioribus tenuibus longisque, ca. 4–5 μ crassis, 4–8 plo diam. longioribus, inflatis in media, sursum gradatim crassioribus brevioribusque, praecipue 2–5 cellulis terminalibus superioribusque fere sphaericis, 10–15 μ crassis; sporangiis unilocularibus plurilocularibusque vel in fronde vel in diversis frondibus productis; sporangiis unilocularibus obovatis vel longe obovatis, 70–85 μ longis, ca. 40 μ crassis; sporangiis plurilocularibus simplicibus rarissime dichotomis,

uniseriatis vel raro biseriatis, fere rectis, crassissimis in media, 6–7 μ diam.; pilis paucis, solitariis, 15–18 μ crassis.

Jap. name: Moku-no-tsuyu (nov.).

Hab.: On leaves of *Sargassum miyabei* in summer.

Frond epiphytic and nearly hemisphaerical, about 0.3–0.6 mm. in diam., slightly penetrating into the host at the base; assimilatory filaments straight, about 170 μ long, consisted of 10–15 cells, lower cells of which thin and long, about 4–5 μ in diam., about 4–8 times as long as diam. inflated at the center, becoming gradually thicker and shorter upwards, especially 2–5 terminal cells nearly sphaerical, 10–15 μ in diam.; both uni- and plurilocular sporangia sometimes produced on the same individual, sometimes on the different individuals, the unilocular ones obovate or long obovate, 70–85 μ long, about 40 μ wide, pluilocular ones simple, very rarely branched uniseriate or biseriate, straight, thickest at the center, 6–7 μ in diam., becoming slightly thinner up- and downwards; hairs few in number, solitary, 15–18 μ in diam.

By having the straight assimilatory filaments with the sphaerical terminal cells, this new species is distinguishable easily from all others of this genus.

Chordariaceae

20. *Sphaerotrichia divaricata f. epiphytica* Inagaki

Pl. 4, B, C.

1954, p. 12; 1958, p. 149, figs. 55, 56, pls. XVI; XVII.

Jap. name: Yase-mozuku.

Hab.: Naka-ban-ya, Sanri-ban-ya and Baro. Epiphytic on leaves of *Zostera* in summer.

Frond gregarious, filiform, flaccid, gelatinous, about 20 cm. long, 1 mm. thick near the base, tapering gradually upwards, provided with a percurrent axis, sending off branches alternately in all directions; branches sending off branchlets repeatedly in alternate manner, both branches and branchlets patent, pliable, often irregularly curled, ramuli often curved; internally at first solid, becoming hollow afterward; assimilately filaments composed of 3–4 cells, 6–12 μ thick; apical cells sphaerical, 29–47 μ high; unilocular sporangia few in number, broadly elliptial; hairs abundant, about 15 μ thick. Colour yellowish brown, turning blackish on drying. Plant adheres closely to the paper.

In the present specimens there are some discrepancies in the sizes of both apical cells and unilocular sporangia in comparison with those in the original description of this form, however, the present ones by having thin and short branchlets coincide with the original description.

Acrothricaceae

21. *Acrothrix pacifica* Okamura et Yamada

Yamada, 1932, p. 113, pl. XXIV, textfig. 2; Okamura, 1936, p. 206, fig. 110; Inagaki, 1954, p. 26, fig. 6, a-c; 1958, p. 178.

Jap. name: Nise-mozuku.

Hab.: Sakae-ura and Cape Kimuaneppu. Epiphytic on *Chorda filum* in summer.

Punctariaceae

22. *Punctaria tenuis* YAMADA and IWAMOTO spec. nov.

Pl. 2, A.

Frons gregaria, flaccida, linearis vel late linearis, breviter stipitata, margine undulata, 13–30 cm, alta, 0.5–7 cm. lata, tenuis, 50–80 μ crassis; cellulis 1–3–stratosis, corticalibus et medullaribus forma et magnitudine aequalibus; cellulis corticalibus superficie 4– vel 5–6–angulatis, 35–60×20–35 μ; sporangiis unilocularibus supra superficies utras frondium sparsis, superficie externe moderate convexis, plurilocularibus ignotis; pilis solitariis. Color pallide fulvis. Frons chartae firme adhaeret.

Jap. name: Usuba-habamodoki (nov.).

Hab.: Sanri-ban-ya and Naka-ban-ya. Epiphytic on leaves of *Zostera*.

Fronds gregarious, flaccid, linear or broadly linear, ending in a short stipe downwards, undulate at the margin, about 13–30 cm. long, about 0.5–7 cm. broad, thin, about 50–80 μ thick, composed of 1–3 layers of cells, both the outer and inner cells alike in the shape and size, the outer ones 4-sided or 5–6-sided in surface view, about 35–60×20–35 μ; unilocular sporangia scattered on both surfaces of the frond slightly bulging on the outer surface, plurilocular ones unknown; hairs solitary, scattered on both surfaces of the frond. Colour pale brown. Frond adheres firmly to the paper in drying.

The new species seems to be rather variable, especially in shape of the frond, some specimens being linear, while the others broadly linear, reaching about 7 cm. in width. There forms, however, are linked by the transitional forms, though there is only a small number of specimens before us. But more hairs are found in the narrow forms than in the broad ones.

The thin frond is a most prominent characteristic of the present species. It does not exceed 80 μ, and we have not met with any portion of the frond where it consisted of above three layers of cells.

The present species, especially the broader form shows some resemblance to *P. latifolia* GREVILLE, but in tne number of the cell layers of which the frond is composed, both species can be easily eistinguished one from the other.

23. *Punctaria kinoshitae* YAMADA and IWAMOTO spec. nov.

Pl. 2, B; Pl. 4, D-F.

Frons gregaria vel solitaria, longe elliptica vel irregulariter expansa, basi late cuneata, breviter stipitata, margine irregulariter undulata et lacerata in speciminibus adultis, hic illic perforata, ca. 17 cm. longa, ca. 10 cm. lata, fragilis, ca. 70–120 μ crassa; cellulis plerumque 2–3-stratosis, saepe hic illic 4-stratosis, forma et magnitudine vel fere aequalibus, vel cellulis medullaribus majoribus; cellulis corticalibus superficie 4–6-angulatis, 40–70×20–40 μ, spor-

angiis unilocularibus supra superficies utras frondium sparsis, superficie externe moderate convexis, plurilocularibus ignotis; pilis pauco aggregatis. Color pallide fulvus, in sicco nigellatus.

Jap. name: Oba-habamodoki (nov.).

Had.: Sanri-ban-ya and Naka-ban-ya. Drifting on bottom in July.

Frond gregarious or solitary ?, long elliptical or irregularly expanded, broadly cuneate at the base ending in a short, about 1 cm. long stipe, in matured specimens irregularly undulate, lacerate at margin, perforated here and there, about 17 cm. long, about 10 cm. wide, fragile, about 70–120 μ thick, composed of usually 2–3, often here and there 4 layers of cells; sometimes both the inner and outer cells alike in the shape and size, but sometimes inner ones larger than the outer ones; cortical cells four to six sided, 40–70×20–40 μ in surface view; unilocular sporangia scattered on both surfaces, moderately bulging on the outer surface; plurilocular sporangia unkown; hairs in small group, scattered on both surfaces of the frond. Colour pale brown, becoming somewhat daker in drying, not adhering to the paper.

The present species seems to be nearly related to *P. expansa* Setchell and Gardner from the Pacific Coast of North America, but in the new species the number of the cell layers of which the frond is composed is smaller than that in the American species, being usually two or three layers though the four layers are often found here and there. Moreover in the species in question no plurilocular sporangia are yet found while in the American species both the unilocular and plurilocular sporangia are produced on the same frond.

24. *Punctaria conglomerata* Yamada and Iwamoto spec. nov.

Pl. 1, B; Pl. 5, A-G.

Frons gregaria vel solitaria, 20 cm. alta, 1–4 cm. lata, 100–120 μ crassa, distorta linearis vel late linearis, stipite breve cum disco parvo, margine irregulariter undulato-crispo, interdum perforata, cellulis medullaribus majoribus; sporangiis unilocularibus supra superficie utras frondium sparsis; sporangiis plurilocularibus ignotis; pilis solitariis vel aggregatis paucis; colore fulvo.

Jap. name : Hida-habamodoki (nov.).

Hab. : Naka-ban-ya to the mouth of the lake. The great quantities of this alga growing on small gastropod shells and small pebbles are drifting on the bottom in July.

Frond gregarious or solitary, up to 20 cm. high, 1–4 cm. wide, 100–120 μ thick, distortedly linear or broadly linear, ending in a short stipe with small disc, with irregularly undulato-crisped margin, sometimes perforated, composed of 3–4 or rarely 2 layers of cells and 1–2 inner cells larger than outer ones in middle part of the frond; unilocular sporangia scattered on both surfaces of frond; plurilocular sporangia unknown; hairs solitary or in small groups; colour yellowish brown.

The new species is characterized by distorted habit and remarkably undulato-crisped margins, but not crenulated along the margins.

Scytosiphonaceae

25. *Scytosiphon lomentarius* (LYNGB.) J. AGARDH
1848, p. 126; HAUCK, 1885, p. 396, fig. 169; OKAMURA, 1908, p. 144, pl. 30; SETCHELL and GARDNER, 1925, p. 531, pl. 44, figs. 72–74; TOKIDA, 1954, p. 104.
Jap. name: Kayamo-nori.
Hab: Sakae-ura and Baro. Growing on pebbles and rocks between the higher and lower tidal belts.

26. *Colpomenia sinuosa* (ROTH) DÈRBES et SOLIER
1856, p. 11, pl. 22, figs. 18–20; OKAMURA, 1907, p. 86, pl. 19, figs. 11, 12; SETCHELL and GARDNER, 1925, p. 539, pl. 45, figs. 82–86; TAYLOR, 1942, p. 51; 1945, p. 83; DAWSON, 1944, p. 232; TOKIDA, 1954, p. 106.
Jap. name: Fukuro-nori.
Hab.: Sakae-ura and Baro. Growing on oyster shells and on *Gymnogongrus*.
Judging from the description of SETCHELL and GARDNER the specimens collected from oyster shells resemble *f. expansa* SAUNDER in having irregularly expanded fronds and the clusters of hairs which are sunk in the tissue. The present ones, however, differ from the form mentioned above by their thin wall and the inner layer composed of 2–4 rows (in *f. expansa* 5–7 rows). Besides, the present specimens also differ from C. *sinuosa f. typica* in the thickness of wall and the clusters of hairs which are sunk in the tissue.

Dictyosiphonaceae

27. *Dictyosiphon foeniculaceus* (HUDSON) GREVILLE
1830, p. 56, pl. 8, figs. 1–4; HARVEY, 1900, p. 164; SETCHELL and GARDNER, 1925, p. 589, pl. 40, figs. 47–49; YAMADA, 1928, p. 507; 1934, p. 14; OKAMURA, 1936, p. 240; KYLIN, 1947, p. 78; TOKIDA, 1954, p. 111.
Jap. name: Uikyo-mo.
Hab.: Sakae-ura and Baro. Epiphytic on *Scytosiphon* in June to August.

28. *Dictyosiphon corymbosum* KJELLMAN
Pl. 1, A; Pl. 4, A; Pl. 13, A-F.
1883, p. 267, pl. 26, figs. 12–15; DE TONI, 1895, p. 447.
Jap. name:
Hab.: Sakae-ura in June.
Some specimens referrable most probably to the present species were found in the same place as *D. foeniculaceus* GREV. In outer appearance they are quite different from the other, and former coincides fairly well with KJELLMAN's description of *D. corymbosum*. The frond becomes hollow in older parts, however, in the character of the unilocular sporangia the difference between the specimens in question and *D. foeniculaceus* is not distinct. And as the writer has identified his specimens without KJELLMAN's so he puts a query after the specific name.
At the top of the branches the writer observed sometimes the hairs in his specimens which were described by REINKE, KUCKUCK etc.

Chordaceae

29. *Chorda filum* (L.) Lamouroux

1813, p. 26; Grevilie, p. 47, pl. VII; Harvey, 1846–'51, pl. 107; Reinke, 1889, p. 62; Setchell and Gardner, 1925, p. 592; Okamura, 1936, p. 243, fig. 134; Kylin, 1947, p. 79; Tokida, 1954, p. 112.

Jap. name: Tsuru-mo.

Hab.: Common in summer.

Sometimes the apical parts of some fronds become hollow and project beyond the water surface.

Fucaceae

30. *Pelvetia wrightii* (Harvey) Yendo

1907, p. 20, pl. I, figs. 4, 5; Tokida, 1954, p. 130.

Jap. name: Yezo-ishige.

Hab.: A floating plant was collected at Tokotan in August.

31. *Sargassum miyabei* Yendo

1907, p. 112, pl. XIV, figs. 13, 14; Nagai, 1940, p. 136; Tokida, 1954, p. 139.

Jap. name: Miyabe-moku,

Hab.: Common along the coast, especially in summer.

This plant is the host of *Myriactula sargassi* and *M. saromaensis*, and has been entangled by *Chaetomorph crassa*, and *Sphacelaria variabilis* grows on the basal part of this *Sargassum*.

32. *Sargassum confusum* J. Agardh

1848, p. 214; Yendo, 1907, p. 106. pl. XIV, figs. 1–12; Tokida, 1954, p. 146.

Jap. name: Fushisuji-moku.

Hab.: ?

A few specimens have been collected.

RHODOPHYCEAE

Bangiaceae

33. *Goniotrichum alsidii* (Zanard.) Howe

1914, p. 75; Inagaki, 1934, p. 12, fig. 5; Yamada and Tanaka, 1944, p. 66; Tokida, 1954, p. 141.

Goniotrichum elegans (Chauv) Zanardini, Rosenvinge, 1909, p. 75, figs. 15, 16; Taylor, 1942, p. 75; 1945, p. 132.

Jap. name: Beni-midoro.

Hab.: Sakae-ura and Baro. Epiphytic on *Zostera* and various algae, e. g. *Polysiphonia*, *Rhodomela*, *Chondrus* and *Sphacelaria* etc. In summer.

34. *Asterocytis ornata* (C. Agardh) Hamel

Pl. 13, G.

1924, p. 451, figs. b-d; Boergesen, 1927, p. 11; Tanaka, 1944, p. 79, figs. 1–3.

Asterocytis ramosa (THWAITES) GOBI, SCHMITZ, 1897, p. 314; WILLE, 1900, p. 7, pl. I, figs. 8–14; ROSENVINGE, 1909, p. 77, fig. 17; BOERGESEN, 1916, p. 3, fig. 1; TAYLOR, 1937, p. 214.
Goniotrichum ramosum (THWAIT.) HAUCK, 1885, p. 519.
Jap. name: Tama-tsunagi.
Hab.: Baro. Epiphytic with *Goniotrichum alsidii*, *Erythrotrichia carnea* and a few *Kylinia moniliformis* on *Polysiphonia japonica*. In summer.

The present specimens distinctly differ from *Goniotrichum alsidii* by the elliptical cells and their green hue, besides the present one has not a tendency of the increase in thickness of the filament near the base as the *G. alsidii*. Judging from the characteristics mentioned above the specimens are closely related with *Asterocytis ornata*. Therefore the writer sought for the reproductive cells in order to identify clearly whether the present ones are belonging undoubtedly to genus *Asterocytis* or not. As far as he has observed the present plants seem to have the equal reproductive cells with those of genus *Goniotrichum*, and the akinete has not been found. But this plant has been found associated with *G. alsidii* on the same host, and so it shows that both plants grow in equal salinity here in spite of the several descriptions stating that *Asterocytis* grows in water of lower salinity than *Goniotrichum* needs (ROSENVINGE, 1909, p. 78). However, at a glance, the present one is distinguishable from *Goniotrichum alsidii* by the filaments elastic in texture.

All characteristics except the nature of the reproductive cell and salinity of water in which our alga grows agree with those of *Asterocytis ornata*, so the writer has identified his specimens with this species.

35. *Erythrotrichia carnea f. tenuis* TANAKA
1944, p. 92, fig. 13.
Jap. name: Hoso-hoshinoito.
Hab.: Sakae-ura and Baro. Epiphytic on *Polysiphonia*, *Rhodomela* and *Sphacelaria* etc. in summer.

Acrochaetiaceae

36. *Kylinia moniliformis* (ROSENVINGE) KYLIN
Pl. 6, E.
1944, p. 16, fig. 9; PAPENFUSS, 1947, p. 437; TAYLOR, 1957, p. 211.
Chantransia moniliformis ROSENVINGE, 1909, p. 99, figs. 28, 29; HOWE, 1914, p.85.
Acrochaetium moniliforme (ROSENV.) BOERGESEN, 1915, p. 22; TAYLOR, 1937, p. 227.
Rhodochorton moniliforme (ROSENV.) DREW, 1928, p. 164; NAGAI, 1941, p. 155, pl. IV, figs. 21, 22.
Chromastrum moniliforme (ROSENV.) PAPENFUSS, 1945, p. 322.
Jap. name:
Hab.: Baro. Epiphytic with *Erythrotrichia* and *Goniotrichum* on *Polysiphonia japonica* in summer.

It has not been able to certify that each cell of the present specimens has a star-shaped chromatophore, however, in other respects the present ones

very well agree with Rosenvinge's description and Kylin's figures of this species.

37. *Acrochaetium daviesii* (Dillwyn) Naegeli

1861, p. 405, figs. 26, 27; Boergesen, 1927, p. 25, fig. 15; Taylor, 1937, p. 234, pl. 31, figs. 1–10; 1945, p. 134; p. 1954, p. 221, pl. 31, figs. 8–10; Kylin, 1944, p. 20, fig. 12; Papenfuss, 1947, p. 435.

Chantransia daviesii (Dillw.) Thuret, in Le Jolis, 1863, p. 106; 1863; Boergesen, 1902, p. 356; Rosenvinge, 1909, p. 104, fig. 34.

Rhodochorton daviesii (Dillw.) Drew, 1928, p. 172; Nakamura, 1944, p. 106, fig. 5.

Jap. name:

Hab.: Sakae-ura. Epiphytic on *Chondrus pinnulatus* and *Gracilaria verrucosa* in the depth of about 2 meters in June.

Squamariaceae

38. *Peyssonelia dubyi* Crouan

Pl. 6, C, D; Pl. 12, E-G.

1844, p. 368; Harvey, 1846, pl. 71; J. Agardh. 1852, p. 501; 1876, p. 384; Hauck, 1885, p. 35; Kuckuck 1897, p. 393, fig. 18; Kylin, 1944, p. 34.

Cruoriella dubyi (Crouan) Schmitz, 1889, p. 20; Rosenvinge, 1917, p. 193 figs. 115–117.

Jap. name:

Hab.: Sakae-ura. Expanding on oyster shells in the depth of about 2 meters in summer.

Thalli membranaceous, horizontally expanding to 3–4 cm. in diam., with protuberances formed by an unusual elongation of cells of erect cell-rows, 210–270 μ thick, and 600 μ more or less in protuberances, firmly adhering to substratum by unicelled and club-shaped rhizoids issued from basal cells; cells of basal layer usually broader than height, 18–48 μ broad, 19–36 μ high; erect cell-rows neary vertical in center of thallus, however, becoming oblique approaching the margin, composed of 7–10 cells, usually higher than broad in lower parts, gradually decreasing in size and transforming to broader than high towards the upper; nemathecia with tetrasporangia elevated above surface of thallus, irregular in outline, attaining to 85–136 μ in height; tetrasporangia being oblong, 70–100 μ high, 30–50 μ broad, terminally produced among the paraphyses composed of 6–10 cells and somewhat curved and not forked; antheridia-stands scattered on upper surface of thallus, 50–83 μ in height; cystocarps unknown. Protuberances having no relation to fructiferous organ.

The present specimens differ from those of Kuckuck in the mode of division of antheridia. But other respects of the former agree with the descriptions and figures mentioned by many investigators.

39, *Rhododermis georgii* (Batters) Collins

Pl. 6, A, B,

1906, p. 160; Tokida, 1934, p. 196; 1954, p, 158; Kylin, 1944, p. 36. *Rhodo-*

dermis van-heurckii HEYDRICH, 1903, p. 246, pl. 17; DE TONI, 1905, p. 1711; KYLIN, 1907, p. 194, fig. 41; ROSENVINGE, 1917, p. 199, figs. 119, 120.
Rhodophysema georgii BATTERS, 1900, p, 377, pl. 414, figs. 8–18.

Jap. name: Fuchitori-beni.

Hab.: Naka-ban-ya. Epiphytic on leaves of *Zostera* in July.

Thalli epiphytic on the edges or rarely on faces of leaves of *Zostera*, horizontally expanded, about 0.5 mm. more or less indiam., 55–115 μ thick; erect cell-rows simple or rarely forked, somewhat loosely combined with each other, composed of 4–8 or rarely 10 cells in full grown portions; cells gradually transforming to higher than broad towards the upper, 5.5–7 μ high, 4–5 μ broad; tetrasporangia teminally produced on erect cell-rows, composed of 3–5 cells, scattered among paraphyses of nemathecia produced here and there on upper surface of thallus, cruciately divided, 30–39 μ long, 20–28 μ broad; paraphyses simple, somewhat curved, composed of 5–7 cells, 40–50 μ tall, about 5.5 μ thick; hairs often arising from apical cells of cell-rows; sexual organ unknown.

In the present species the large parenchymatous cells, which were stated by HEYDRICH, COLLINS, KYLIN and ROSENVINGE, were not observed. However there are two forms growing on a same leaf of *Zostera*, the one is the comparatively flat form, the other is the non-flat one, and such a difference in the thickness of thalli is due to the number of cells consisting the vetical cell-rows of the inner tissue.

Concerning the tetrasporangia which are terminally produced on the erect cell-rows of the thalli, the present specimens agree with the ROSENVINGE's description and figures of *Rhododermis elegans* (p. 197, fig. 118 D, 1917) and in this respect the present ones differ from *R. georgii*. In other respects Saroma's specimens perhaps suggest the disc-shaped form of *Rhododermis georgii*.

Corallinaceae

40. *Fosliella farinosa* (LAMOUROUX) HOWE
1920, p. 587; TAYLOR, 1937, p. 270; 1942, p. 91; 1957, p. 252.
Melobesia farinosa LAMOUROUX, FARLOW, 1881, p. 180.
Jap. name:
Hab.: Common with *Fosliella lejolisii* on leaves of *Zostera* in summer.

41. *Fosliella lejolisii* (ROSANOFF) HOWE
1920, p. 588; TAYLOR, 1937, p. 270, pl. 36, figs. 6–8; 1942, p. 91; 1957, p. 253, pl. 36, figs. 6–8.
Melobesia lejolisii ROSANOFF, FARLOW, 1881, p. 180; KJELLMAN, 1883, p. 105.
Jap. name:
Hab.: Common on leaves of *Zostera*.

42. *Corallina pilulifera* POSTEL and RUPRECHT
Pl. G, H.
1840, p. 20, pl. XL, fig. 101; RUPRECHT, 1850, p. 344; KUETZING, 1858, pl. 64; YENDO, 1902, p. 10, pl. III, figs. 14–16, pl. VII, figs. 14–16; TOKIDA, 1954, p. 161.

Jap. name: Pirihiba.
Hab.: Horoiwa, Maruyama, Tokotan and Baro. Growing on rocks.

Grateloupiaceae

43. *Grateloupia divaricata* Okamura
1895, p. 482, pl. 9, figs. 1, 2; 1913, p. 55, pls. 116, 117, figs. 12–18; Tokida, 1954, p. 162.
Jap. name: Kata-nori.
Hab.: Horoiwa, Maruyama. Tokotan and Baro. Growing on rocks.

Endocladiaceae

44. *Gloiopeltis furcata* (Postel and Ruprecht) J. Agardh
1851, p. 235; Okamura, 1927, p. 164; Tokida, 1954, p. 163.
Jap. name: Fukuro-funori.
Hab.: Tokotan. Growing on rocks.
In Lake Saroma this species reaches to hardly 1 cm. in height.

Gracilariaceae

45. *Gracilaria verrucosa* (Hudson) Papenfuss
1950, p. 195; Tokida, 1954, p. 174; Taylor, 1957, p. 273, pl. 36, fig. 1; Ohomi, 1959, p. 6, figs. 1, 2, pl. I, A-D.
Fucus verrucosus Hudson, 1762, p. 470.
Gracilaria confervoides (L.) Greville, 1830, p. 123; Harvey, 1846, pl. 65; Okamura, 1916, p. 1, pl. 151; Dawson, 1944, p. 295; 1949, p. 13, pl. 15, fig. 9.
Jap. name: Ogo-nori.
Hab.: Common along the coast, especially at Sakae-ura and Baro.

Phyllophoraceae

46. *Gymnogongrus japonicus* Suringar
1870, p. 36, pl. 24, A; De Toni, 1897, p. 248; Okamura, 1921, p. 181, fig. 9; 1936, p. 644.
Jap. name:
Hab.: Baro. Growing on rocks.
Judging from Okamura's figures of *G. flabelliformis* the present specimens suggest to be more closely related to *G, japonicus* than *G. flabelliformis* by their rather winding and elongated branches, however, the branching of the present one is of comparatively regular dichotomy as *G. flabelliformis* is.

Gigartinaceae

47. *Chondrus pinnulatus* (Harvey) Okamura
1930, p. 19, pl. 263, figs. 1–6
Gymnogongrus pinnulatus Harvey, 1856, p. 332; Martens, 1866, p. 133; De Toni, 1897, p. 253.

Jap. name: Hira-kotoji.

Hab.: Sakae-ura. Growing on oyster shells in the depth of 2 meters. Baro and Tokotan. Growing with *Grateloupia* and *Gymnogongrus* on the rocks.

Rhodymeniaceae

48. *Chrysymenia wrightii* (HARVEY) YAMADA

1932, p. 118, pl. XXV, textfig. 4.

Halosaccion wrightii HARVEY, 1859, p. 332,; DE TONI, 1895, p. 42; 1900, p. 608.

Chylocladia wrightii OKAMURA, 1916, p. 51.

Chrysymenia enteromorpha YENDO (non HARVEY), 1917, p. 85.

Jap. name: Taoyagi-so.

Hab.: Sakae-ura. Growing on oyster shells in the depth of 1–2 meters in spring to summer.

The present specimens agree well with the detailed description of the author of this species. The young plants collected in June have the distinct axes attaining to 4–6 cm. in height, and the axes are unbranched, however, send off the numerous spine-like branchlets to every directions. The antheridia bearing plant has not been collected.

Champiaceae

49. *Lomentaria hakodatensis* YENDO

Pl. 14, F; Pl. 15, F, G.

1920, p. 6; DAWSON, 1944, p. 308; TOKIDA, 1954, p. 195.

Jap. name: Kosuji-watsunagi-so.

Hab.: Baro. Epiphytic on *Gymnogongrus* in July.

All specimens which have been collected are male, they are 4–5 cm. in height and about 0.5 mm. in diameter at the lower parts, and the internodes are 4–5 mm in length. Many gland cells, which are produced on the face of the inner cavity, are observed at each node of frond, they have the diameter of 7–14 μ. The antheridia are produced in groups on the surfaces of the middle parts of fronds.

Ceramiaceae

50. *Antithamnion sparsum* TOKIDA

1932, p. 105, textfigs. 1, 2, pl. III a; 1954, p. 196.

Jap. name: Kinuito-yotsugasane.

Hab.: Sakae-ura. Growing on oyster shells in summer.

A few specimens which grow with *Antithamnion corticatum*, *Ceramium cimbricum* and *Colpomenia sinuosa* on the oyster shells in the depth of 1.5 meter, were collected in July. The reproductive organs were not found, however, the habit agree quite well with that of *A. sparsum* collected and described by Prof. J. TOKIDA from lagoon Tobuchi in Saghalien.

51. *Antithamnion corticatum* Tokida
1932, p. 108, textfigs. 3–5, pl. III, b-d; 1954, p. 198.
Jap. name: Beni-hanemo.
Hab.: Sakae-ura. Growing with *A. sparsum* on shells.

52. *Ceramium cimbricum* Petersen
Pl. 7.
1923, p. 378, figs. 318, 319; Tokida, 1948, p. 100, figs. 10-28; 1954, p. 202.
Jap. name: Matsubara-igisu.
Hab.: Sakae-ura. Growing with *Antithamnion sparsum* and *Ant. corticatum* on oyster shells in the depth of 2 meters, and on *Ceramium kondoi* and *Chorda filum* in July and August.

Prof. J. Tokida has described and figured the specimens of this species from Kaiba Island and Lake Tobuchi in southern Saghalien, and all of his specimens were tetrasporic. The tetrasporangia of Saroma's specimens quite agree with Tokida's description and figures in the mode supporting them at the nodes and their dimensions. Besides the writer often observed in the present plants that the tetrasporic plants produce the cystocarps, and tetrasporangia are produced also on the cystocarpic plants, while on the antheridial plants any of them have not been observed.

The specimens, which have been collected from the oyster shells, have had the well-developed creeping filaments, and the internodal cells of the creeping ones are often filled with grains showing purplish hue. On the contrary the specimens found on *Chorda* are solitary and provided with very short creeping parts or wanting them entirely.

53. *Ceramium kondoi* Yendo
Pl. 8, A, B.
1920, p, 9; Nakamura, 1950, p. 160, figs. 4, 5; Tokida, 1954, p. 202.
Jap. name: Kondo-igisu.
Hab.: Sakae-ura, Baro and Naka-ban-ya. Growing on shells, pebbles and rocks.

Dasyaceae

54. *Dasya sessilis* Yamada
1928, p. 524, fig. 19, a-c; Inagaki, 1934, p. 70, figs. 30, 31.
Jap. name: Enashi-dajiya.
Hab.: Sakae-ura. Growing on shells washed by the tidal current in August. A few small and sterile plants growing on rocks were collected at Baro in the same season.

Rhodomelaceae

55. *Polysiphonia japonica* Harvey
Pl. 8, C; Pl. 15, E.
Gepp. 1904, p. 163, pl. 460, figs. 4–6; De Toni, 1924, p. 393; Yamada, 1928, p. 522; Segi, 1951, p. 228, pl. 8, fig. 3; Tokida, 1954, p. 219.

Jap. name.: Kiburi-itogusa.

Hab.: Sakae-ura and Baro. Growing on pebbles and rocks, and on Chorda.

The plants found on gravelly bottom are caespitose, and their erect filaments adhere frequently to each other by the rhizoids. Solitary plants found on the rocks and on *Chorda* issue several procumbent filaments at their basal parts.

The writer occasionally observed that two tetrasporangia emptied by the liberation of tetraspores are filled by the small cells. Besides the writer has observed three times that the antheridia-like small cells have been produced on the upper parts of pericarp of the aborted cystocarps.

Saroma's specimens differ from the previous descriptions of *P. japonica* in the present of the rhizoids in erect filaments, but the external appearance and other characteristics agree well with this species.

56. *Polysiphonia urceolata* GREVILLE

Pl. 6, F; Pl. 8, D.

1824, p. 309; HARVEY, 1846-'51, pl. 167; 1853, p. 32; KUETZING, 1849, p. 924; 1863, pl. 92, figs. a, b; J. AGARDH, 1863, p. 970; ARDISSONE, 1883, p. 397; HAUCK, 1885, p. 221; FALKENBERG, 1901, p. 150; DE TONI, 1903, p. 875; YENDO, p. 60; YAMADA, 1928, p. 522, figs. 17; OKAMURA, 1936, p. 824, figs. 388, 389, SEGI, 1951, p. 239, pls. 10, 11, figs. 26, 27; TOKIDA, 1954, p. 219.

Jap. name: Shojo-kenori.

Hab.: Sakae-ura. Growing on shells in summer.

The specimen agrees with *P. urceolata* in general appearance, the form of the cystocarp found in the former, however, differs from that of the latter, that is, cystocarps of the former are almost globose in ripe, young ones, however, are urceolate in form.

In the lowermost part of the frond the numerous spine-like branchlets are issued uniseriately, and that is owing the remarkable torsion of the pericentral cell-rows; besides the articulations of the lower part remarkably swell at the junctions.

The present specimens may be identified also with *P. fibrata* by having globose cystocarps with largely opened ostioles, the swelling at the junctions and remarkable torsion in the lower filaments. But this plant differs clearly from *P. fibrata* in external appearance.

Male plant has not been found.

57. *Polysiphonia elongata f. schuebelerii* (FOSLIE) ROSENVINGE?

Pl. 9, F-H.

1923, p. 419, figs. 361, 362.

Jap. name:

Hab.: Sakae-ura. Epiphytic on leaves of *Zostera* in August.

Fronds up to 7 cm. in height, solitary, rigid, yellowish brown, often issuing the procumbent filaments from the bases, pseudo-dichotomously branching in the main branches, with alternate or unilateral ramuli; filaments attenuated towards the upper, being thickest at the first or second branching parts, up to 450 μ in diam.; pericentral cells four in number, not corticated,

those of the middle and upper parts often complicated by one another; articulations visible throughout the frond, 1.5–2 times or more as broad in the middle part, diminishing the length equal to their breadth in the ramuli, and shorter than their breadth near the base, some procumbent filaments adhere to substratum by the attaching organ, and growing upwards and becoming the erect filaments; tetrasporangia borne in swollen and distorted ramuli, or in branchlets issued from the main filaments; male and female plants unknown.

In the present specimens it is very conspicuous that the complicated pericentral cells are very often recognized in the middle and upper parts of the fronds as Rosenvinge has observed (Mar. Alg. Denmark, 1923–'24, p. 419, fig. 361). This complication irregularly occurs among cells in an articulation and between the articulations, besides these complicated pericentral cells rarely cut off the small cells.

58. *Chondria dasyphylla* (Woodward) C. Agardh

Pl. 14, A-E.

1822, p. 350; 1824, p. 205; Harvey, 1853, p. 20; Falkenberg, 1901, p. 197; Kylin, 1907, p. 138; Boergesen, 1918, p. 258, figs. 151, 152; Okamura, 1936, p. 863; Dawson, 1944, p. 325; Tokida, 1954, p. 218.

Jap. name: Yanagi-nori.

Hab.: Sakae-ura and Naka-ban-ya. Growing on leaves of *Zostera* in August.

59. *Chondria polyrhiza* Collins and Hervey

Pl. 9, A-E; Pl. 15, B-D.

1917, p. 121, pl. II, fig. 12.

Jap. name;

Hab.: Sakae-ura. Growing gregariously on the bottom composed of pebbles, sand and shells in the lower tidal belt in summer.

The plants have two kinds of the filaments, the one is normal erect filament, and the other is entangled one, which is found in the lower part of frond and is thinner than the erect one. The entangled ones issue the unicellular rhizoids in groups here and there, besides issue the hapters composed of many cells, and by those the entangled ones and erect ones adhere to each other.

The growing apices are remarkably protruded. In the lowermost parts of the main filaments the central cells are often replaced by the masses of the smalls rhizoidal cells, and the pericentral cells are connected with them. Rosenvinge described in *Polysiphonia elongata* that the central cells sometimes were filled by the hypha-like filaments (Mar. Alg. Denmark). In general appearance the present specimens seem to be nearly related to *Ch. tenuissima*, but in the present ones the filaments are thinner and firmer than those of the latter, besides the former's filaments adhere very often to each other by the rhizoids and hapters mentioned above. So the writer referred the present specimens to *Ch. polyrhiza*, though the present ones have not any subdentately outlined branches as the original plants have.

60. *Laurencia nipponica* YAMADA

Pl. 10, A-C.

1931, p. 209, pl. 9; TOKIDA, 1954, p. 216.

Jap. name: Ura-sozo.

Hab.: Growing on rocks in summer. The male plant has not been found.

61. *Symphyocladia latiuscula* (HARVEY) YAMADA

1941, p. 215

Jap. name: Iso-murasaki.

Hab.: Sakae-ura. Growing on the lowermost parts of *Sargassum miyabei* in Augst.

The present specimens have no reproductive organs.

62. *Rhodomela larix* (TURNER) C. AGARDH

Pl. 10, D-F; Pl. 15, A.

HARVEY, 1853, p. 24; FALKENBERG, 1901, p. 600, pl. XI, fig. 1; OKAMURA, 1922, p. 154, pl. 188, figs. 1–4; TOKIDA, 1954, p. 221.

Jap. name: Fuji-matsumo.

Hab.: Growing on rocks.

Some specimens resemble *R. subfusca* in general appearance, but the others agree well with *R. larix* in cartilaginous texture and rather vigorous main branches. And both types are connected by the transitional forms. Therefore the writer has identified his specimens with this species. In some tetrasporic plants two forms of branches have been observed in the same individual, one is vigorous branch which spirally issues the penultimate branches issuing a large number of the incurved tetrasporangia bearing branchlets, the other is thinner and rather flexible one which issues a numerous tetrasporangia bearing branchlets without the penultimate branches as in the former, these branchlets are simple or sometimes branched, and they are remarkably incurved and slightly distorted. The tetrasporangia which are accompanied with the large cover cells in pairs are produced in a double rows in the branchlets mentioned above. The cystocarps are produced on the small branches. The antheridial bodies have not been found.

The list of marine algae from Lake Saroma and their distribution in various districts in Hokkaido and its vicinity.

	Mutsu Bay	Okushiri Isl.	Oshoro Bay	Robben Isl.	Southern Saghalien	Shiretoko Pen.	Shikotan Isl.	Kurile Islands	Urup Isl.	Akkeshi Bay
CHLOROPHYCEAE										
1. *Enteromorpha intestinalis*		+			+	+		+		+
2. *Ent. compressa*	+	+								

	M.	Ok.	Os.	R. I.	S. S.	S. P.	S. I.	K.	U.	A.
3. *Ent. plumosa*					+		+	+		
4. *Ent. crinita*										
5. *Ent. linza*	+	+			+		+	+		+
6. *Ulva pertusa*	+	+			+	+	+	+		+
7. *Monostroma zostericola*		+			+		+	+	+	+
8. *Chaetomorpha crassa*	+									
9. *Cladophora stimpsonii*	+				+					
10. *Spongomorpha saxatilis*	+						+	+		
11. *Bryopsis plumosa*	+	+								
PHAEOPHYCEAE										
12. *Ralfsia verrucosa*					+					
13. *Ascocyclus orbicularis*										
14. *Halothrix tortuosa*										
15. *Sphacelaria variabilis*	+				+					
16. *Dictyota spathulata*	+									
17. *Leathesia difformis*	+	+			+	+		+		+
18. *Myriactula saromaensis* spec. nov.										
19. *Myriactula sargassi*					+					
20. *Sphaerotrichia divaricata f. epiphytica*										
21. *Acrothrix pacifica*		+			+	+		+		
22. *Punctaria tenuis* spec. nov.										
23. *Punct. kinoshitae* spec. nov.										
24. *Punct. conglomerata* spec. nov.										
25. *Scytosiphon lomentarius*	+	+		+	+		+	+	+	+
26. *Colpomenia sinuosa*	+	+			+		+	+	+	+
27. *Dictyosiphon foeniculaceus*	+				+	+	+	+	+	+
28. *Dict. corymbosum*										
29. *Chorda filum*	+	+			+	+		+		
30. *Pelvetia wrightii*	+				+	+	+	+		+
31. *Sargassum miyabei*		+			+	+		+		+
32. *Sarg. confusum*	+	+			+			+		
RHODOPHYCEAE										
33. *Goniotrichum alsidii*		+	+		+	+				+
34. *Asterocytis ornata*										
35. *Erythrotrichia carnea*					+					+
36. *Kylinia moniliformis*									+	
37. *Acrochaetium daviesii*										
38. *Peyssonelia dubyi*										
39. *Rhododermis georgii*					+					
40. *Fosliella farinosa*										

	M.	Ok.	Os.	R. I.	S. S.	S. P.	S. I.	K.	U.	H.
41. *Fosl. lejolisii*										
42. *Corallina pilulifera*	+				+			+		+
43. *Grateloupia divaricata*	+	+	+		+	+				+
44. *Gloiopeltis furcata*	+	+	+		+	+	+	+		+
45. *Gracilaria verrucosa*	+		+	+	+	+	+	+		+
46. *Gymnogongrus japonicus*										
47. *Chondrus pinnulatus*				+	+	+	+	+		+
48. *Chrysymenia wrightii*	+	+	+							
49. *Lomentaria hakodatensis*	+	+	+		+	+				+
50. *Antithamnion sparsum*					+					
51. *Ant. corticatum*					+					
52. *Ceramium cimbricum*					+					
53. *Cer. kondoi*		+			+			+		+
54. *Dasya sessilis*	+		+							
55. *Polysiphonia japonica*	+	+	+		+	+	+	+		
56. *Poly. urceolata*	+	+	+		+					+
57. *Poly. elongata f. schuebelerii*										
58. *Chondria dasyphylla*	+				+	+				+
59. *Ch. polyrhiza*										
60. *Laurencia nipponica*		+	+		+			+		
61. *Symphyocladia latiuscula*		+								+
62. *Rhodomela larix*		+	+		+	+	+	+		+

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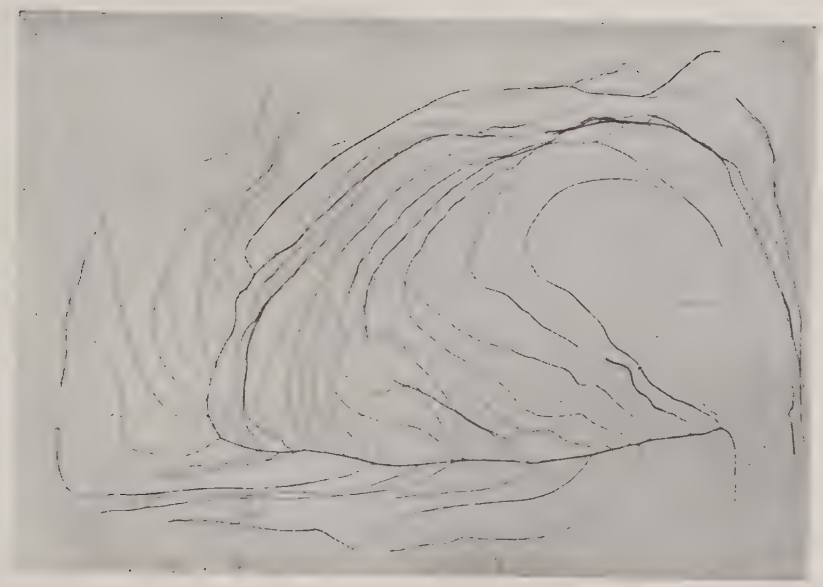
PLATES I–XV

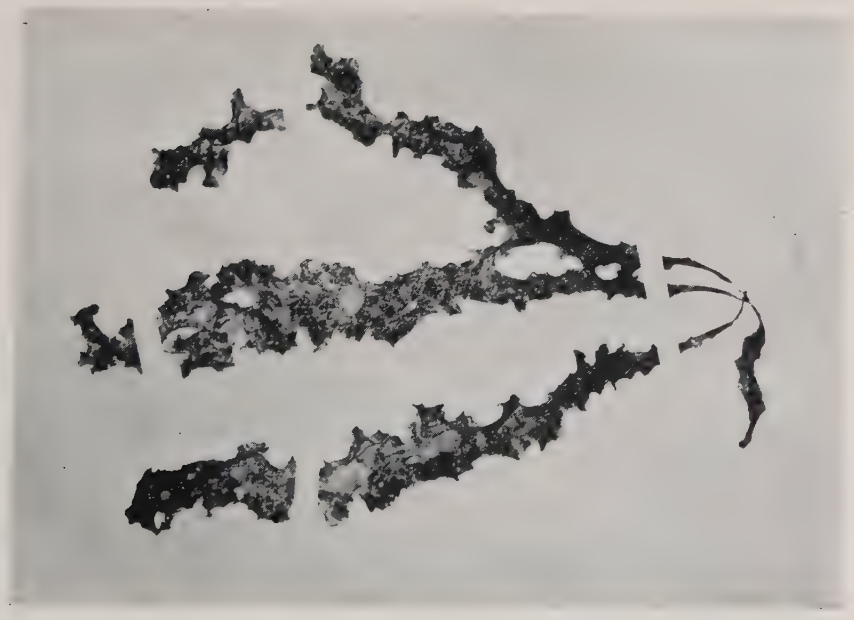
PLATE I

A. *Dictyosiphon corymbosum* KJELLMAN. ×2/5.

B. *Punctaria conglomerata* YAMADA and IWAMOTO spec. nov. ×2/3



A.



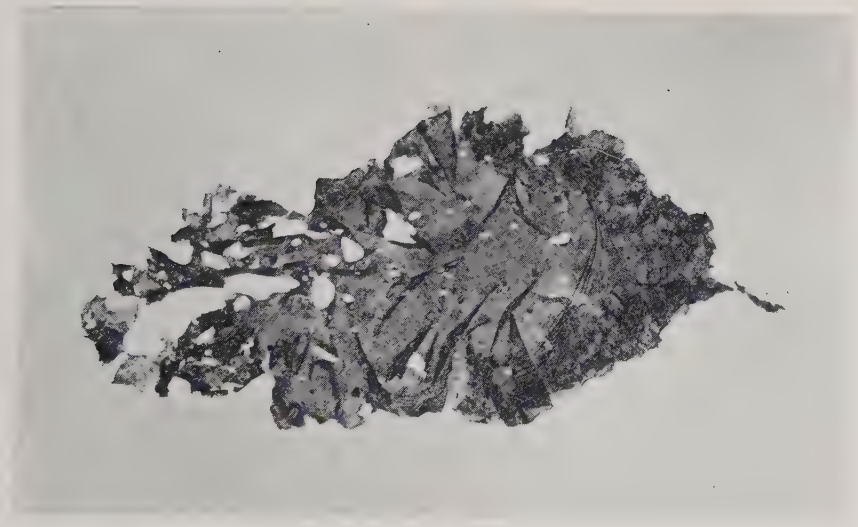
B.

PLATE II

A. *Punctaria tenuis* YAMADA and IWAMOTO spec. nov. ×2/5.

B. *Punctaria kinoshitae* YAMADA and IWAMOTO spec. nov. ×3/5

A.



B.

PLATE III

Myriactula saromaensis YAMADA and IWAMOTO spec. nov.

A. Vertical section through the center of a gametangia bearing plant. ×140.
B. Gametangia, assimilatory filaments and a hair. ×440.
C. Gametangia and assimilatory filaments. ×440.
D. Branched gametangia.
E. Sporangium and assimilatory filaments. ×440.

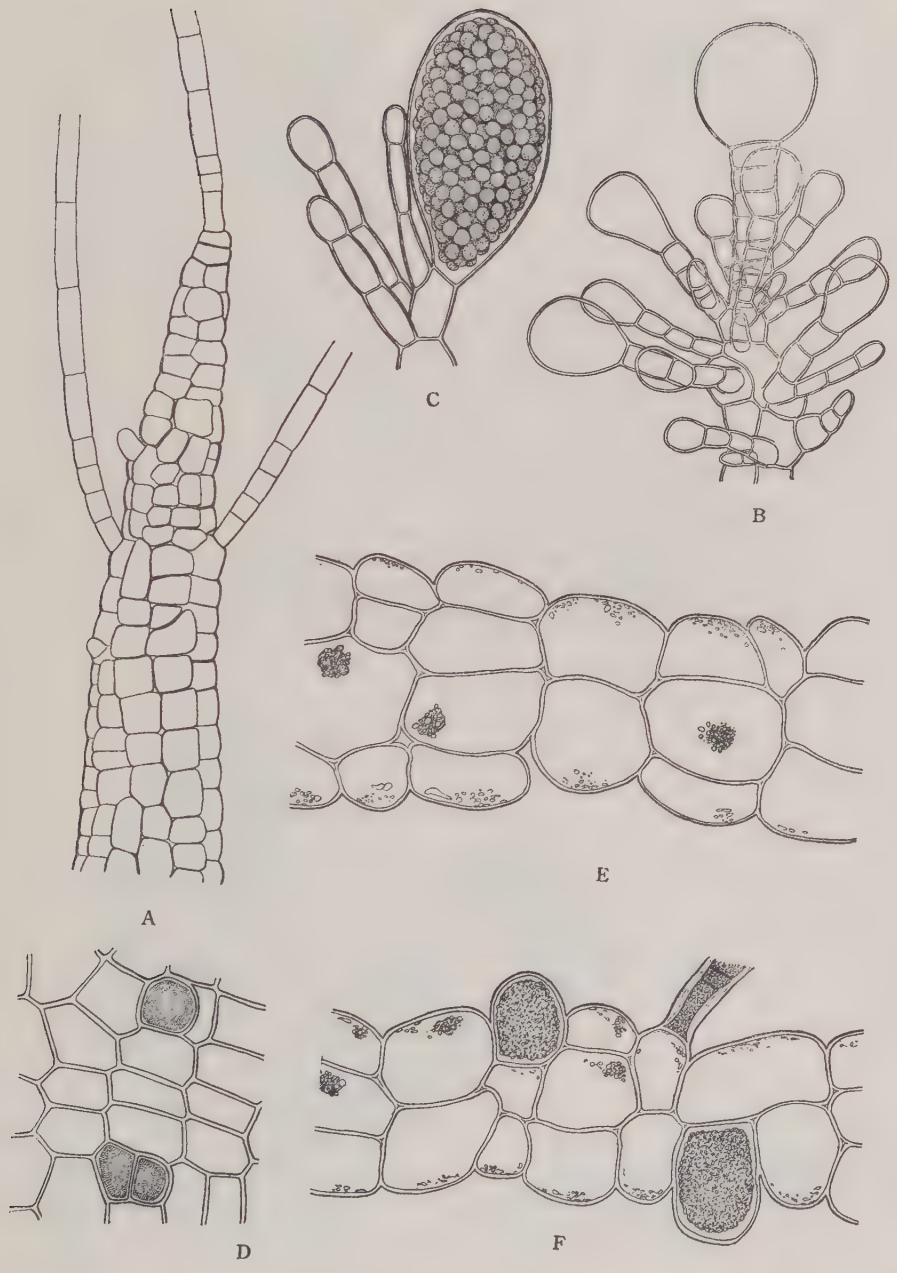
B
D
E
C
A

PLATE IV

A. *Diotyosiphon corymbosum* KJELLMAN.
Tip of a filament issuing three hairs. ×540.

B, C. *Sphaerotrichia divaricata* f. *epiphytica* INAGAKI.
B. Tip of a filament. ×540.
C. A unilocular sporangium. ×540.

D-F. *Punctaria kinoshitae* YAMADA and IWAMOTO spec. nov.
D. Surface view of a frond, showing three unilocular sporangia. ×160.
E. Cross section through the four layers and two layers portions. ×270.
F. Cross section through the two unilocular sporangia. ×270.


A
C
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E
D
F

PLATE V

Punctaria conglomerata YAMADA and IWAMOTO spec. nov

A-C. Various habits of fronds. ×5/6.
D. Cross section through a sporangium. ×160.
E. Cross section, showing two inner and two outer layers. ×160.
F. Cross section, showing a group of hairs. ×160.
G. Cross section of a free marginal portion. ×160.

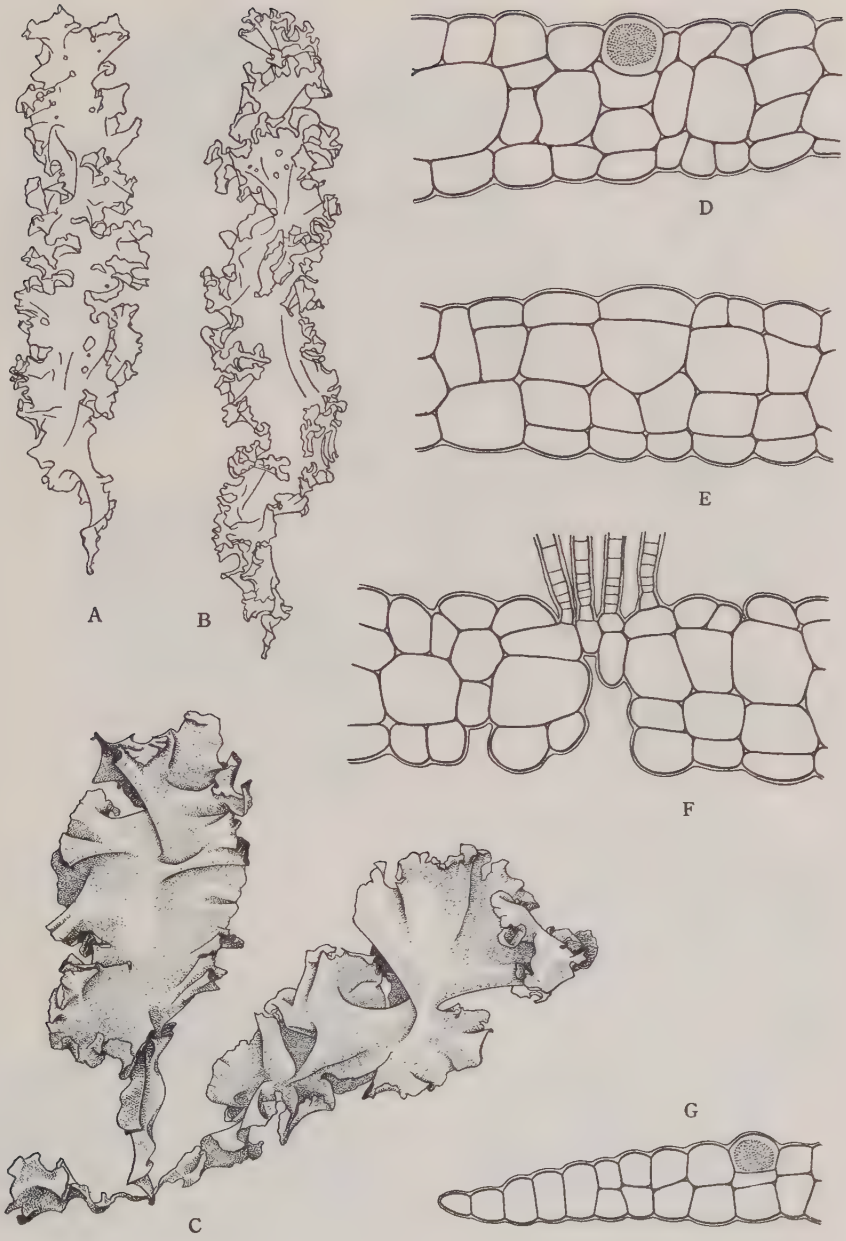
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PLATE VI

A, B. *Rhododermis georgii* (BATTERS) COLLINS.

A. Vertical section of a tetrasporic plant. ×270.

B. A part of a basal layer seen from the under. ×540.

C, D. *Peyssonelia dubyi* CROUAN.

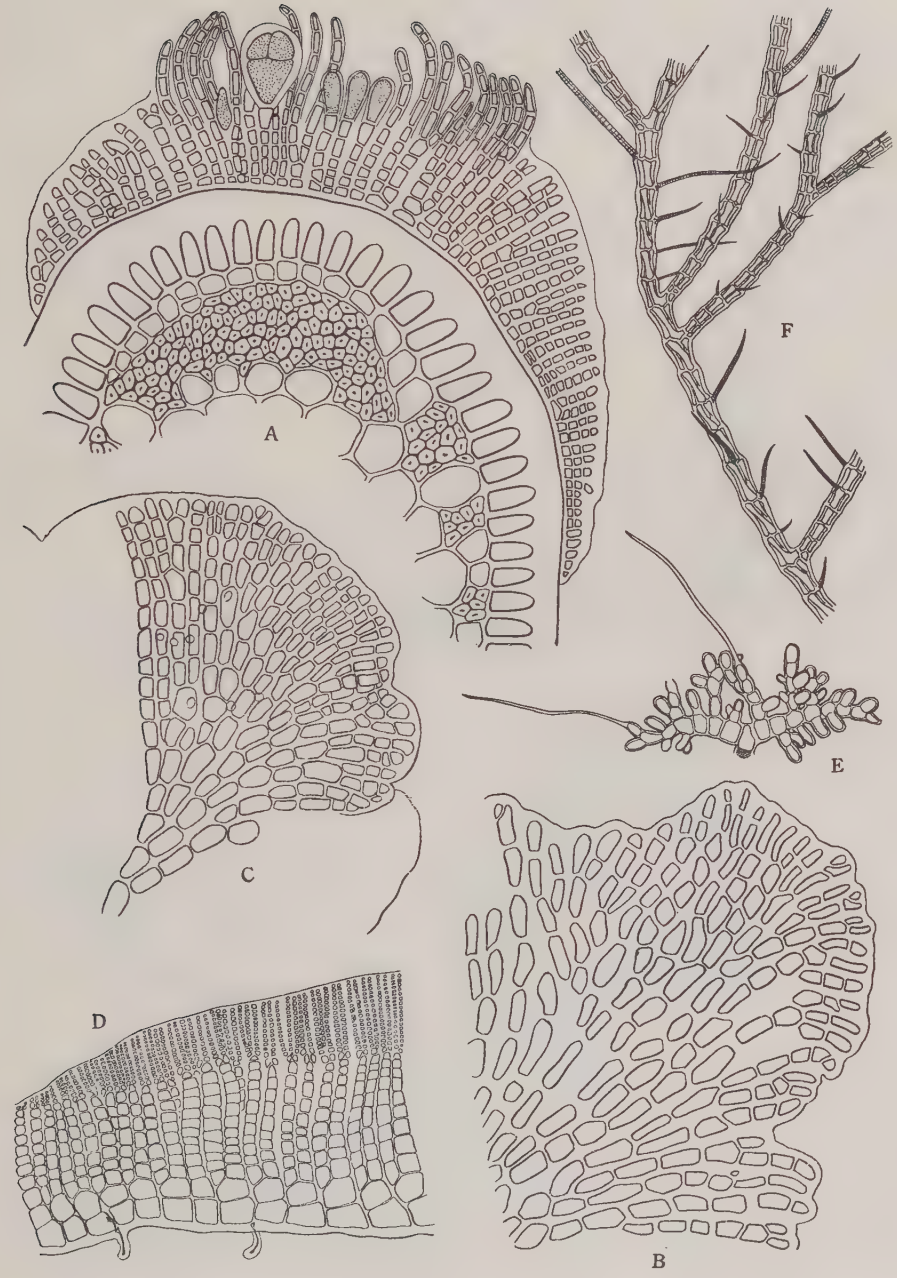
C. A part of a basal layer seen from the under, showing several unicellular rhizoids. ×160.

D. Vertical section through an antheridial stand. ×160.

E. *Kylinia moniliformis* (ROSENVINGE) KYLIN. ×440.

F. *Polysiphonia urceolata* GREVILLE.

Lower part of a frond. ×16.


A
F
C
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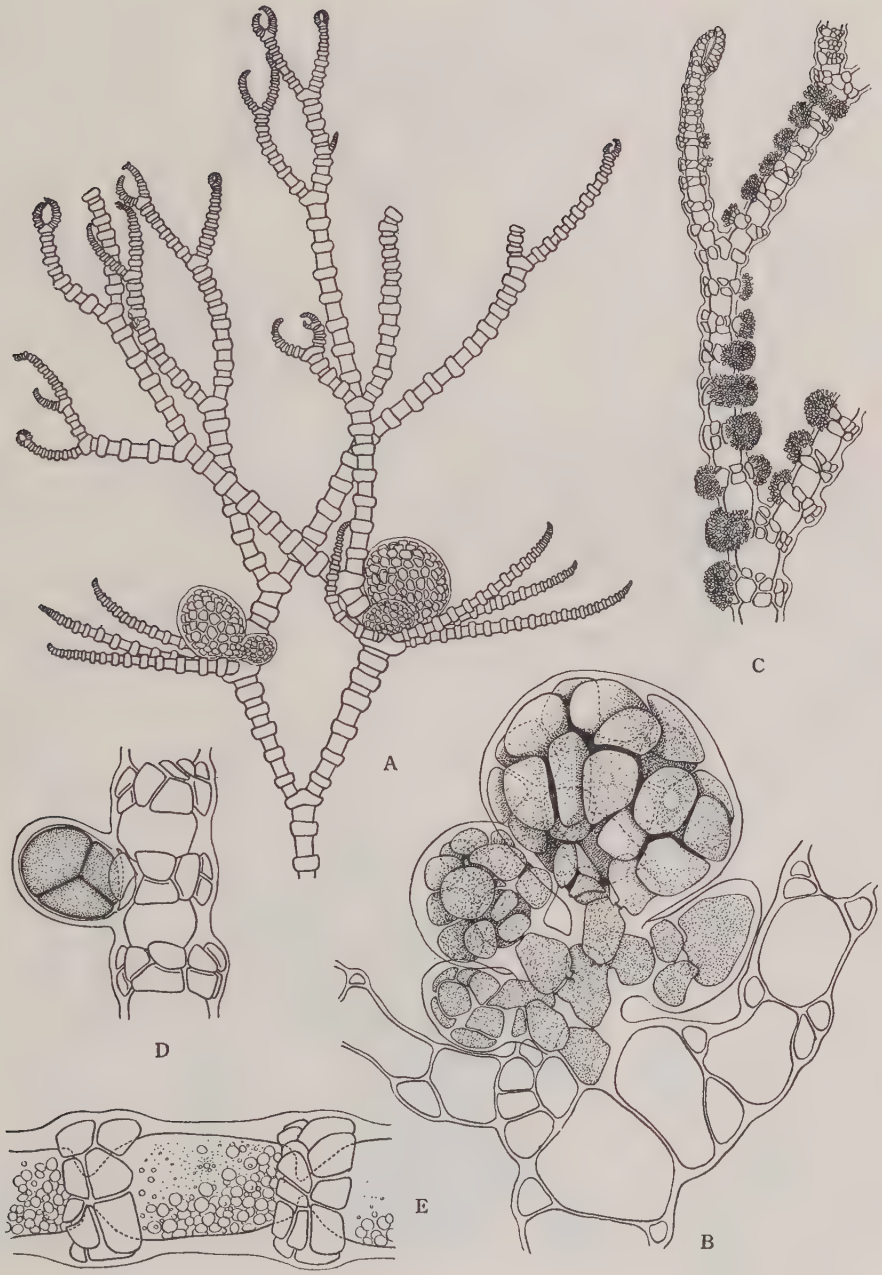
D
B

PLATE VII

Ceramium cimbricum PETERSEN.

A. Habit of an upper branching of a carposporic plant, showing cystocarps and involucral branchlets. ×27.
B. Cystocarps. ×270.
C. Upper folks of a spermatangial plant. ×80.
D. Tetrasporangium. ×270.
E. Cells of the creeping filament, they contain many grains. ×27.


A
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E

PLATE VIII

A, B. *Ceramium kondoi* YENDO.
A. Habit of an upper branching of a carposporic plant. ×6.
B. Habit of an upper branching of a tetrasporic plant. ×6.
C. *Polysiphonia japonica* HARVEY.
Habit of a branching of a tetrasporic plant. ×6
D. *Polysiphonia urceolata* GREVILLE.
Habit of a ramuli with tetrasporangia. ×16.

A
B
C
D

PLATE IX

A-E. *Chondria polyrhiza* COLLINS and HERVEY.

A. Habit of an upper part of a tetrasporic plant. ×6.
B. Habit of an entangled filament. ×6.
C. Tip of an entangled one issuing the several rhizoids. ×50.
D. Tissued hapter issued from an entangled filament, ×90.
E. Central rhizoidal cells connecting with the pericentral cells. ×160.

F-H. *Polysiphonia elongata* f. *schuebelerii* (FOSLIE) ROSENVINGE ?.

F. Middle part of a filament, showing the intricated pericentral cells. ×50.
G, H. Cross sections of the intricated articulations. ×90.

A
B
C
D
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F
G
H

PLATE X

A-C. *Laurencia nipponica* YAMADA.

A. Habit of an upper portion of a carposporic plant. ×6.

B. Habit of an upper portion of a tetrasporic plant. ×6.

C. Cross section of a filament, showing the thickening cell walls. ×28.

D-F. *Rhodomela larix* (TURNER) C. AGARDH.

D. Habit of a branching from a tetrasporic plant. ×6.

E. Other form of a tetrasporangia bearing branch. ×6.

F. Habit of a branching from a carposporic plant. ×6.

A
E
D
F
B
C

PLATE XI

Myriactula sargassi (YENDO) FELDMANN.

Vertical section through the center of a gametangia bearing plant growing on the vesicle of Sargassun miyabei. ×160.

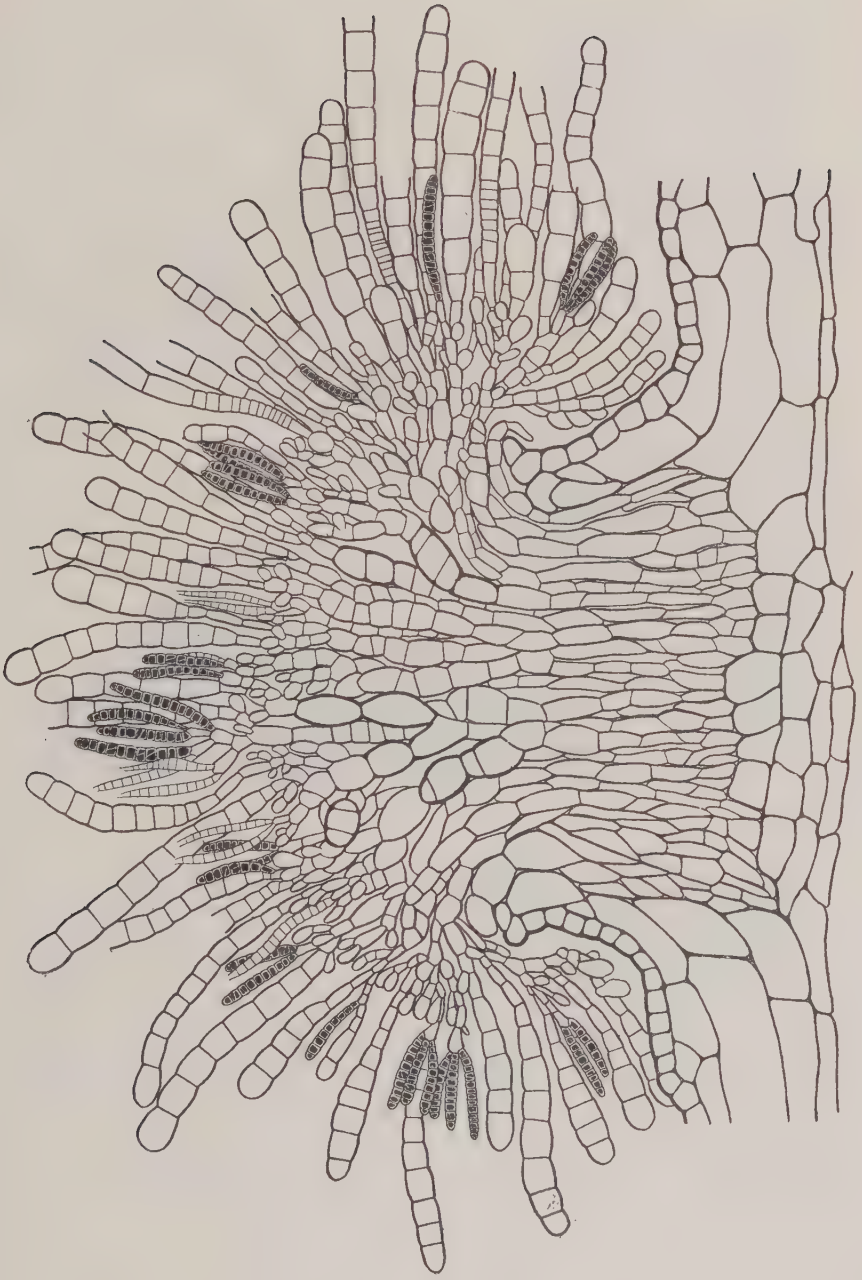

PLATE XII

A, B. *Ascocyclus orbicularis* (J. AGARDH) MAGNUS.

A. Section showing the plurilocular gametangia, asci and a hair. ×540.

B. Marginal portion of a frond seen from the under. ×540.

C, D. *Halothrix tortuosa* TAKAMATSU.

C. Young plurilocular gametangia. ×270.

D. Matured plurilocular gametangia. ×270.

E-G. *Peyssonelia dubyi* CROUAN.

E. Section through the protuberance. ×45.

F. More magnified basal part of the figure shown in E. ×270.

G. Section through tetrasporangia. ×160.

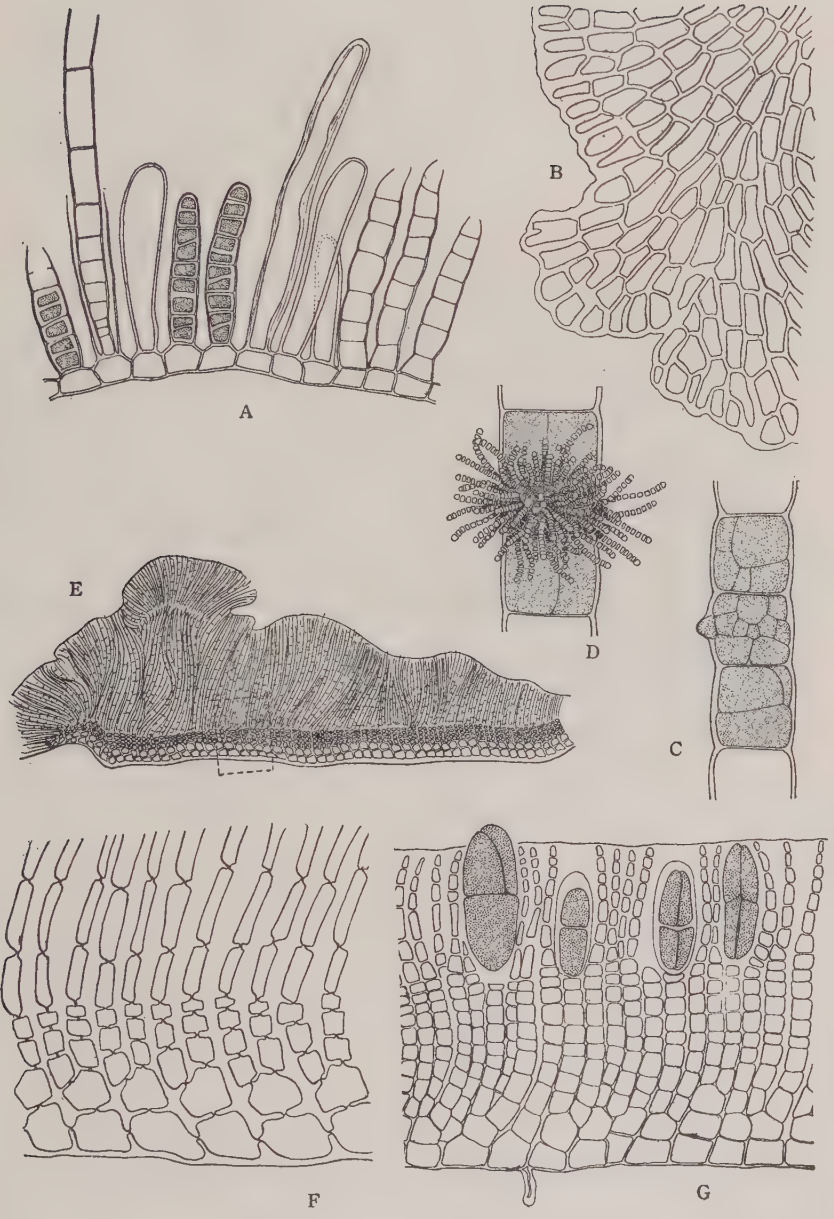
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PLATE XIII

A-F. *Dictyosiphon corymbosum* KJELLMAN.

A. Cross section of the upper part of a filament. ×270.

B. Cross section of the middle part of a filament. ×270.

C. Cross section of the lower part of a filament. ×160.

D. A part of the branch producing the monosporangia. ×45.

E. Inner large cells facing the cavity and showing three monosporangia ×160.

F. Cross section through the monosporangia. ×250.

G. *Asterocytis ornata* (C. AGARDH) HAMEL.

False branching. ×160.

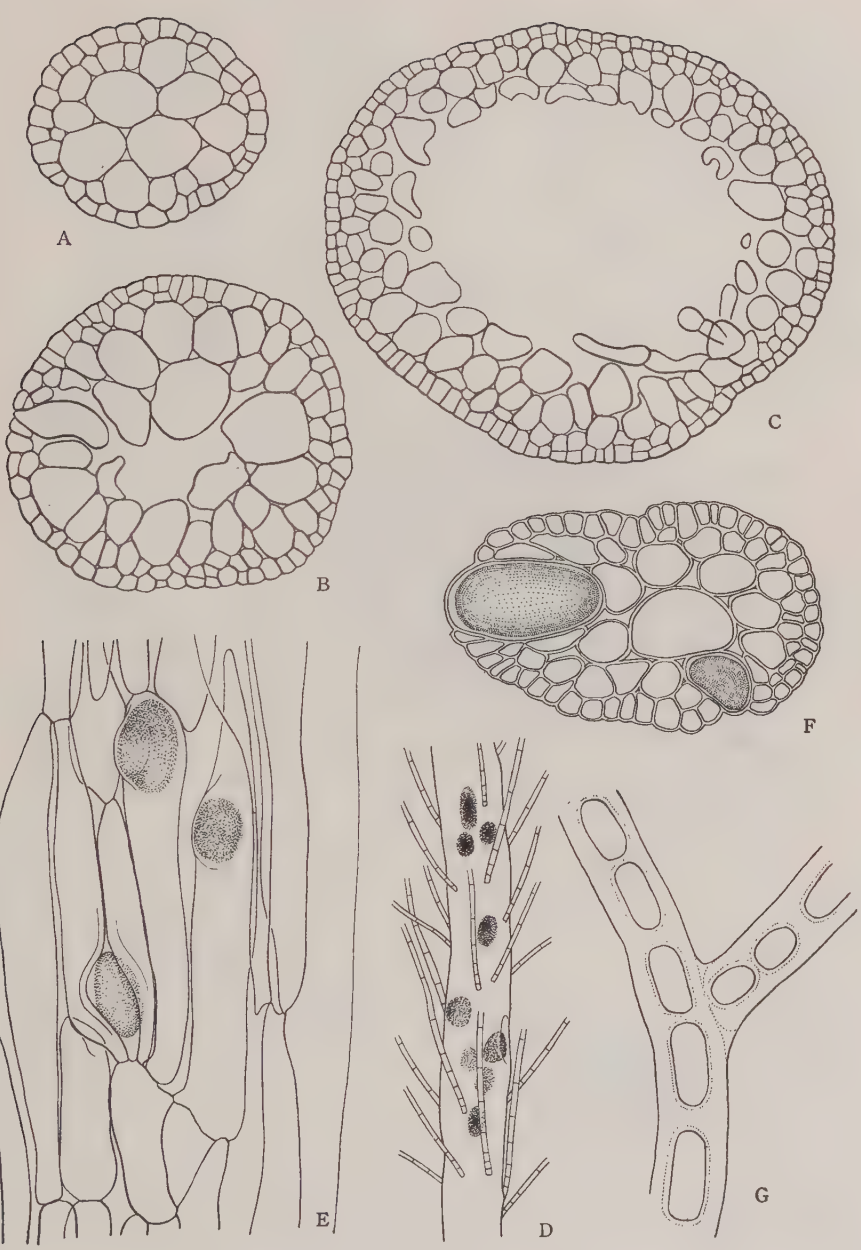
A
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PLATE XIV

A-E. *Chondria dasyphylla* (WOODWARD) C. AGARDH.

A. A portion of a frond producing the antheridial bodies. ×8.

B. Young antheridial body with a trichoblast. ×270.

C. A riped antheridial body. ×90.

D. Antheridia and their supporting cells. ×540.

E. A portion of a frond producing the cystocarps. ×8.

F. *Lomentaria hakodatensis* YENDO.

F. Surface of a frond, densed cells are dotted. ×540.

G, H. *Corallina pilulifera* POSTEL and RUPRECHT.

G. A cystocarpic branch. ×16.

H. A tetrasporic branch. ×16.

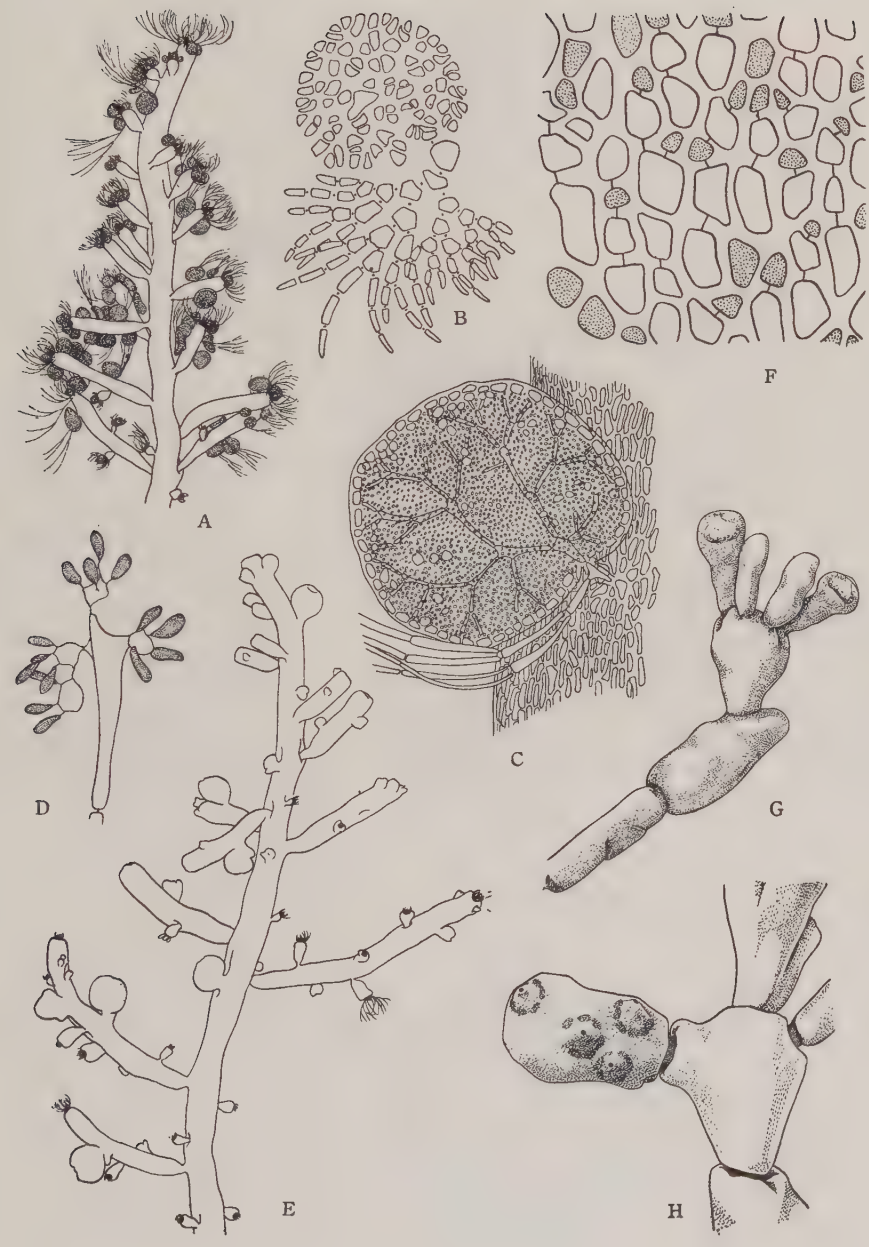
A
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PLATE XV

Rhodomela laix (TURNER) C. AGARDH.

Tetrasporangia bearing branchlet. ×160.

B-D. *Chondria polyrhiza* COLLINS and HERVEY.

B. Surface view of a tetrasporangium with two cover cells. ·160.

C. Cross section of an upper part of a filament. ×250.

D. Cross section of a lowermost part of a filament. ×45

E. *Polysiphonia japonica* HARVEY.

Antheridia produced on the pericarp of aborted cystocarp. ×90.

F, G. *Lomentaria hakodatensis* YENDO.

F. Masses of the gland cells produced at a node. ×27

G. Two gland cells. ×540.

A
B
E
F
C
D
G

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STUDIES ON THE GLYCOLYSIS AND THE FORMATION OF AMMONIA IN THE MUSCLE AND BLOOD OF ELASMOBRANCHS

Michizo Suyama, Junzo Koike and Kazuo Suzuki

(Received Feb. 15, 1960)

The glycogen contained in the muslce of animals is dissolved into lactic acid with their movement and it is recreated by their repose, while it has long been known that in the anaerobic metabolism of glycogen proceeding after the death of animals glycogen is dissolved straight into lactic acid and preserved in muscle. Nowadays, metabolites relating to this glycolysis have been made clear one after another, and reaction system of glycolysis has been almost established. Many researches are going on regarding the glycolysis of fish muscle[1~13], too, and the change of pH value, glycogen, lactic acid, etc., the influence of their fatigue and the influence upon the quality of fish muscle given by method of fishing or by treatment of fish after the fishery . . . these are all getting clarified gradually.

In such researches, needless to say, comparison or obsevation of the results of test will lack precision unless the method of treatment and the time lapse after the death of fish are clearly given; the researches by Amano *et al*[9] and Fujimaki *et al*[10], who, paying particular attention to this point, recently put before us detailed data about frigate mackerel, make it clear that frigate mackerel fished by angling shows marked anaerobic metabolism by a few minutes violent shock after its struggling, that, from the view of distribution of phosphorylated metabolite, glycolysis mostly comes near to an end, while the fish instantly killed by a proper means retains much vital element in its body[14], and that moreover the glycolysis in such fish muscle is considerably slow.

As to the glycolysis of muscle of elasmobranchs, Yamada *et al*[5] made comparative researches on *Mustelus manazo* and *Heterodontus japonicus* caught by trawl-net and long-line, respectively. These researches have so far revealed that *Heterodontus japonicus* requires more time than *Mustelus manazo* to reach maximum formation of lactic acid, which result is supposed to come from the difference in fishing method between the two kinds of fish. As for the glycolysis of the other kinds of elasmobranchs, however, there is not a piece of research as yet.

One of the authors reported the quantities of urea and ammonia contained in the muscle or many organs of fresh-caught shark and ray[15], where it was found that even in the muscles immediately after death the content of ammonia nitrogen amounted to as high as 6.7 to 24.2 mg%. Of course, such a great percentage of ammonia is not physiological, but it may be considered a result caused by some violent shock at the time of catching. Fujimaki *et al*[10] assumed that in the muscle of frigate mackerel, when inosinic acid is formed from adenosine monophosphate by deamination, ammonia is to isolate itself, and perhaps the formation of ammonia arising from the similar reaction system may be the reason for the foregoing result of 6.7 to 24.2 mg% ammonia-nitrogen in the muscle of

fresh-caught elasmobranchs.

From such a point of view, the authors have made a series of researches on the glycolysis of elasmobranchs with particular care about the quantity of ammonia contained, and these researches went on very favorably with a shark and ray kept in the water-tank of the aquarium for a certain time. Below are given the details of the test in three parts—glycolysis in muscles, in bloods, and rise and fall of phosphorylated metabolites in muscles and livers.

PART 1. RISE AND FALL OF THE AMOUNTS OF ACID SOLUBLE PHOSPHORYLATED METABOLITES AND THE FORMATION OF AMMONIA IN MUSCLE WITH FATIGUE

The formation of ammonia arising from glycolysis is not peculiar to elasmobranchs. It has already been observed in muscle of ordinary fish[9-10], but the former fish, unlike the latter, remains alive long after fishery. Therefore, some characteristic results are expected to be obtained as to reaction of glycolysis of the muscle of elasmobranchs.

In Part 1 the experimental results on elasmobranchs as to how the amount of acid soluble phosphorylated compounds will change by fatigue, and how it will cause the formation of ammonia, are shown.

Experimental and Results

Test fish and method of treatment of its body

Sharks live usually at or near the bottom of the sea and can be fished by trawl net, set net, angling or the like, but fishes caught by any of these methods are so fatigued that they cannot be used as rest fishes for test. It was necessary that those fishes, caught by a usual means, make recovered from the fatigue while they are kept in a water-tank.

As test material, several fishes such as *Triachis scyllium* M. & H. and *Dasyatis akajei* (M. & H.) which were caught along the coast of Kominato and thereafter fed with salted mackerel pikes for about two weeks in the water-tank of the Kominato Laboratory Aquarium, were used for.

At first the authors used a *Dasyatis akajei*, 28 cm. in length which was scooped in a net without making it fatigued and then immediately killed by repeated hammerings on its head. The muscle obtained was analysed for acid soluble phosphorylated compounds, and in this test an *Oplegnathus fasciatus* (T. & S.) was also used for comparison.

Next, two *Triachis scyllium* M. & H. of the same sex (♀) and the same size (72 cm. in length) were killed for experiment; one of which was immediately killed like the foregoing *Dasyatis akajei* M. & H. while the other was botheringly poked in the water-tank for about 30 minutes and then was hammered on the head to death. By this the authors tested the difference between the two in the amounts of acid soluble phosphorylated compounds formed in the muscles while preserving each piece of muscles at 14°C. for 4 hours, and in cracked ice for 5

days, in the course of which muscles were picked up to be compared in respect of the rise and fall of phosphorylated compounds.

The test fish in the group of instant killing stopped moving by being hammered on its head, with its muscles convulsing now and then. As to the group of fatigue, the authors tried to kill the fish by leaving it on, the floor of the laboratory, but such treatment failed to cause the fish to violently moved to death, so the fish was put in a seawater-tank in which the fish was forced to swim by poking on head or tail. This treatment made the fish move around restlessly and 10 minutes later the fish came to be markedly fatigued, but when stimulation was stopped, the fish quickly recovered its liveliness and would hardly die. Therefore the authors drove the fish for 30 minutes on end, and when the fish become not to move a bit even if rolling its body upside down, it was clubbed to death and was used for the test.

Method of Analysis

Two pieces of muscles of 2 g. and 7 g. each were weighed by a chemical balance, then the 2 g. piece was put into a test tube which had already been filled with 30% alkaline solution and put in boiling water to be warmed and dissolved. After this, while cooled down in cracked ice, it was carried back to the laboratory, and was determined of the amount of glycogen. Another piece of 7 g. muscle was put into a test tube which had beforehand been filled with 10 cc. of 10% trichloracetic acid and fully ice-cooled, carried back also to the laboratory, cooled down there as before, and was determined of the amounts of acid soluble phosphorylated compounds.

The analysis of acid soluble phosphorylated compounds was followed by the method of LePage[16]. That is, 40 cc. of ice-cooled 5% trichloracetic acid was added to the test muscles, blended with a blender, and centrifuged. With the supernatant fluid thus obtained, it was determined the amounts of creatine phosphate (CP) and lactic acid as soon as possible, and separated the remaining percolation into barium insoluble and soluble fractions. With the former fraction, the amount of inorganic phosphoric acid, Δ7P, adenosine triphosphate (ATP), adenosine diphosphate (ADP), hexose diphosphate (HDP), and phosphoglyceric acid (PGA), were determined, and as for the latter fraction, it was further separated into alcohol insoluble and soluble fractions, and then with the former fraction, the analysis was made on the amounts of inorganic phosphoric acid, glucose-1-phosphate (G1P), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), triose phosphate (TP), phosphopyruvic acid (PPA), ribose phosphate (RP), and adenosine monophosphate (AMP), and the optical densities at 260 mμ and 248 mμ to represent adenylic acid and inosinic acid, respectively. With the alcohol soluble fraction, the amount of total phosphorus was determined. These calculations of phosphorus in phosphorylated compounds were all followed by Fiske-Subbarow method[17].

Distribution of acid soluble phosphorylated compounds in the muscles of *Dasyatis akajei* and *Oplegnathus faciatus*

Table 1. Distribution of acid soluble phosphorylated compounds in the muscle of *Dasyatis akajei* and *Oplegnathus fasciatus*

	Dasyatis akajei	*Oplegnathus faciatus*
pH	7.03	6.90
Ammonia N	3.60	3.28
Lactic acid	112	456
Glycogen	230	576
Total phosphate P	194	218
Inorganic phosphate P	97.1	120
True inorganic phosphate P	82.8	112
CP-P	14.3	8.4
Barium insoluble fraction		
Total phosphate P	111	148
Inorganic phosphate P	77	102
△7P	12.5	29.5
ATP-P	4.0	25.2
ADP-P	19.6	25.4
HDP-P	8.8	9.1
PGA-P	0	0
Barium soluble alcohol insoluble fraction		
Inorganic phosphate P	16.8	17.8
G1P-P	0.6	0.9
G6P-P	42.6	29.0
F6P-P	2.8	9.0
AMP-P	8.0	11.1
TP-P	0.3	2.9
PPA-P	0	0
RP-P	0.5	0.6
Barium soluble alcohol soluble fraction		
Total phosphate P	5.4	7.2

In Table 1 are shown the results of determination of acid soluble phosphorylated compounds in the muscles of *Dasyatis akajei* and *Oplegnathus fasciatus* which were scooped in a net out of the water and instantly killed by clubbing.

It was shown that pH value was higher with *Dasyatis akajei* (7.03) than with *Oplegnathus fasciatus* (6.90). Formation of lactic acid was much higher with *Oplegnathus fasciatus* than with *Dasyatis akajei*, and this will have caused the above difference in pH value between the two. As to the amount of ammonia nitrogen, there were little or no difference between the two, both showing slightly over 3 mg%. Glycogen was considerably low in *Dasyatis akajei*, and this may be considered one of the characteristics of elasmobranchs, as stated below.

Distribution of acid soluble phosphorylated compounds shows a much greater proportion of G6P besides ATP and ADP, and this G6P seems to be the axis of balance in cycle of glycolysis through the process of glycogen→G1P→G6P→F6P→HDP→TP→PPA→lactic acid and in the regeneration of glycogen through the reversed process. As to the content of each phosphorylated compound, there were found

more or less difference between the two kinds of test materials, i. e., *Dasyatis akajei* is higher in CP and G6P while *Oplegnathus fasciatus* higher in ATP, ADP, AMP and TP. These metabolites usually change according to quantity of motion of fish, so there cannot argue the difference in glycolysis between the two immediately from the foregoing results.

Rise and fall of the amounts of acid soluble phosphorylated compounds and formation of ammonia arising from fatigue

Of the two *Triakis scyllium*s of the same sex (♀) and size (72 cm. in length) bred in a water-tank under the same conditions, one was killed instantly and the other by clubbing after making it completely fatigued, and then were determined the amount of ammonia and acid soluble phosphorylated compounds in the muscle of each fish. In Table 2 are given the results of this and of the test on rise and fall of the above mentioned compounds in the course of 4-hours storage of the muscles at 14°C. and 5-days storage in cracked ice.

In this test the muscle of an instantly killed *Triakis scyllium* did not come to rigor mortis even 4 hours later, while the muscles of the group of fatigue proved to go into evident rigor mortis 2 hours later. This fact suggests that there is a distinctive difference in process of glycolysis between the two groups, and its evidences are shown in Table 2.

In the group of instant killing, pH value rans 6.70 at first, then gradually declined to 6.21 after 24 hours, showing a decrease of 0.49, while in the group of fatigue it showed a value of 6.28 from the beginning, then very gradually lowered to 6.04 after 24 hours, showing a decline of 0.24. Based on the figure 6.70 for the group of instant killing, the portion as much as 0.66 changed into acid side. For reference, pH value at the time of rigor mortis was 6.18.

Lactic acid content in the muscle of group of instant killing ranged from 415 mg% to max. 1,240 mg% in 24 hours and then showed a slight decline, while in the group of fatigue it was retained 1,240 mg% from the outset and finally max. 1,520 mg%. Glycogen content is higher in the group of instant killing than in the other, but it seems to be lower than the analyzed result of *Oplegnathus fasciatus* shown in Table 1. This glycogen gets gradually resolved while the muscles are stored, and is almost lost after 24 hours and on, but in the group of fatigue it is very low from the beginning. However, the fact that in this group the amount of glycogen showed a slight increase in 2 to 4 hours after death is not found in the group of instant killing.

As to ammonia nitrogen content there is a great difference between the two groups, i. e., 3.43 mg% for the group of instant killing and 9.84 mg% for the other. In both groups ammonia nitrogen content increased during the preservation of the muscles, and this is, needless to say, not due to the decomposition of the muscle but to the deamination of adenylic acid or the like mentioned below. *At least the fact that when the fish moves violently ammonia content in the muscles increases is worthy of notice.*

Of acid soluble phosphorylated compounds, it is indicated that CP remains in a good deal in the group of instant killing, while it is scarcely found in the

Table 2. Rise and fall of the amounts of acid soluble phosphorylated compounds

Sample	Time hr.	pH	Ammonia N	Lactic acid	Glycogen	Total phosphate P	Inorganic phosphate P	True inorganic phosphate P	CP–P	Barium insoluble			
										Total phosphate P	Inorganic phosphate P	△7P –P	ATP –P
Group of instant killing	0	6.70	3.43	415	332	196	128	117	11	133	99	26.3	20.6
	1	6.57	4.05	653	262	212	131	121	10	143	103	21.1	11.0
	2	6.51	6.11	975	172	222	154	150	4	142	119	15.9	6.2
	4	6.39	7.83	1140	29	218	158	151	5	153	129	12.1	3.0
	24	6.21	12.9	1240	9	222	163	160	3	140	128	8.2	2.8
	72			1130	0	216	175	175	0	152	146	3.3	1.4
	120			1110	0	227	184	184	0	161	153	0	0
Group of fatigue	0	6.28	9.84	1240	37	201	137	136	1	127	110	10.4	5.0
	1	6.18	11.3	1280	26	205	136	135	1	125	110	9.0	0.5
	2	6.18	11.4	1380	48	212	136	136	0	132	121	8.2	1.4
	4	6.06	11.8	1520	45	212	137	137	0	129	120	3.3	0
	24	6.04	14.9	1510	10	185	151	151	0	127	126	2.9	4
	72				7		156	156	0				
	120			1520	3	198	164	164	0	138	132	1.3	0

group of fatigue. Such is also the case with ATP and ADP, total content of which amounts to two times that of the group of fatigue. ATP declines as glycolysis proceeds, while that decline is covered by the increase of ADP at the beginning, together by the increased AMP and inosinic acid. Such process has been well indicated. In the group of fatigue it was found much AMP and inosinic acid from the beginning, which tells that the method of causing fatigue adopted in the test was a considerably rigid one.

HDP and PGA are retained in low quantities, and the difference in them between the two groups is not clear. Of six phosphates of the monosaccharide, G6P was highest in amount, and especially in the group of instant killing it increased by and by with time and finally reached max. 46 mg%.

Putting all the above test results together, it was assumed that glycolysis is still going in the group of instant killing, while in the group of fatigue the reaction system has almost come to an end.

Observations

As stated before, glycogen level seems much lower in elasmobranchs than in other ordinary fishes. AMANO *et al*[9] reported glycogen content in the muscles of frigate mackerel to be 1,000 mg%; in this research *Oplegnathus fasciatus* contained 576 mg% glycogen, while *Dasyatis akajei* had only 230 mg% and *Triakis*

and formation of ammonia arizing with fatigue in muscle of *Triachis scyllium* (mg%)

fraction			Barium soluble alcohol insoluble fraction										Barium soluble alcohol soluble fraction Total phosphate P
ADP −P	HDP −P	PGA −P	Inorganic phosphate P	G1P −P	G6P −P	F6P −P	AMP −P	T P −P	PPA −P	RP −P	E_{248}*	E_{260}*	
17.7	5.5	0	19.2	0	6.7	4.0	6.0	1.1	0	2.1	0.230	0.178	6.0
24.6	0	0.7	25.6	0.5	17.5	5.8	9.3	2.0	0	0.8	0.370	0.276	5.4
23.6	0	0	28.9	1.3	17.5	7.2	15.4	0.1	0	1.0	0.504	0.456	6.3
35.6	0	0	21.7	1.0	18.0	9.4	17.4	0	0	0.4	0.761	0.517	6.2
12.6	0	0	25.9	0.9	45.8	4.0	21.4			0	0.972	0.636	6.0
4.7	0	2.5	20.3	0.4	20.5	2.5	20.4			0	0.820	0.606	5.9
0	0	6.2	19.3	0	20.5	2.3	22.3			0	0.670	0.664	5.4
13.6	0	0	21.2	1.2	17.4	3.1	19.0	0	0	0	0.840	0.566	5.8
17.3	3.2	0	20.5	3.1	10.9	3.2	17.5	0	0	0.4	0.823	0.519	6.6
14.5	0	0	20.8	1.4	16.9	3.7	19.6	0	0	0	0.858	0.583	6.6
6.6	0	0.3	20.7	2.1	19.1	3.0	21.4	0	0	0	0.955	0.635	7.2
5.8	0	0	17.8	1.2	18.5	3.8	22.9			0	1.060	0.680	7.3
2.5	0	7.1	17.6	0.8	13.3	3.9	16.9			1.4	0.772	0.504	5.8

* Test was made on a solution equivalent to 0.04g./cc. muscles.

scyllium 332 mg%. Out of 1,000 mg% glycogen is created about 1,100 mg% lactic acid, and glycogen, if traced back on this basis from the foregoing lactic acid content, amounts to about 330 mg% in *Dasyatis akajei* and about 700 mg% in *Triakis scyllium*. Elasmobranchs, however, contains only little glycogen in their muscles, while they hold thick glycogen in their livers which are distinctively larger than those of other fishes (the livers used in this test contained 1,950 mg% glycogen), and taking it into account that this thick glycogen moves into muscles from the livers while the fish is moving, we are led to assume that, in general, elasmobranchs, too, belongs to those animals which contain a high level of glycogen. So, it is also comprehensible that the created quantity of lactic acid amounts to 1,200 to 1,500 mg%.

As a results of such a great creation of lactic acid, pH value in the muscles of ordinary fishes tends to acid. Frigate mackerel showed max. 1,280 mg% lactic acid, and had rigor mortis at pH 5.6[9], also in whale meat, the final figure of pH value at which lactic acid was created in enough quantity is said to run near 5.7[18]. But *Triakis scyllium* is slow in decline of pH value even when the created lactic acid amounts to nearly the same degree, and it should be regarded quite different from frigate mackerel in that it went into rigor mortis at pH 6.18. Among the reasons for this difference will be found the trimethylamine oxide content in the muscles. This trimethylamine oxide, which shows no buffer-

ing capacity on the alkaline side but has a strong buffering capacity on the acid side[19], is thickly distributed in the muscles of elasmobranchs, and this may control the decline of pH. On the other hand the opening period of rigor mortis has been said to be affected directly by the remaining ATP content[20], and no portion of ATP was found in the test fish of the group of fatigue in 4 hours after the rigor mortis began. This result conforms with those of past researches.

The physiological concentration of ammonia cannot be mentioned cleary from the above test alone, and the best interpretation seems to be that it will be 3 mg% or less in the form of ammonia nitrogen. This figure was found to run over 10 mg% at the time of fatigue, and it can also be explained that ammonia content of 6.7 to 24.2 mg% in the fresh muscles immediately after the catch[14] has been created in connection with the intense glycolysis at the time of fishery which is accompanied by a particular long-time violent shocks in the case of using long-line, trawl nets, etc. For a similar phenomenon appearing in frigate mackerel, FUJIMAKI et al[10] have been pointed out the provable reason in deamination of amide like glutamine and of adenylic acid. In this research the authors examined the extinctions of absorption spectrum at 260 mμ representing adenylic acid and at 248 mμ representing inosinic acid which produced by deamination in all the samples. As given in Table 2, the optical densities at 260 mμ and 248 mμ go up with time in the group of instant killing, and run up very high from the beginning in the group of fatigue. The optical density at 248 mμ always exceeded that at 260 mμ. From this fact it is induced that a great deal of inosinic acid is preserved, and that accordingly in proportion to it much ammonia also is formed.

Summary

Using a *Dasyatis akajei* and a *Triakis scyllium* bred in a water-tank under a certain condition, the distribution of acid soluble phosphorylated compounds and ammonia, and the rise and fall of these materials and their change during the preservation have been examined.

(1) Glycogen content is low in elasmobranchs, but an inflow of liver glycogen to muscle during the motion of fish may cause an intense glycolysis to create a lot of lactic acid.

(2) A clear change was found in those various metabolites from glycogen to lactic acid and CP, ATP, and ADP related to the transfer of energy.

(3) When the muscle of *Triakis scyllium* entered into the rigor mortis, pH value of muscles showed 6.18, there existing not a bit of ATP.

(4) As to ammonia content in the muscle, 3 mg% or less as existing in the form of ammonia nitrogen was considered a phsyological concentration, but it was found that by fatigue or preservation of muscles the figure rises to 10 mg% or more (max. 15 mg% has been proved by this test). A portion of this increased ammonia is thought to due to be deamination of adenylic acid forming in the latter half of glycolysis.

PART 2. RISE AND FALL OF THE AMOUNTS OF ACID SOLUBLE PHOSPHORYLATED COMPOUNDS AND FORMATION OF AMMONIA IN BLOOD THROUGH FATIGUE.

In a usual physiological state liver glycogen goes into blood glucose, and creates muscle glycogen. Lactic acid formed from this glycogen by glycolysis is partly oxidized and decomposed, but largely carried back with blood to the livers in which it re-creates glycogen. If there is such a violent motion, however, liver glycogen is carried to the muscles in the form of blood glucose and gives a birth to lactic acid, which is likely to be delayed in changing into glycogen and stay in blood.

There is no reference regarding the determination of blood of fish at the time of glycolysis, but in the blood perhaps much lactic acid and various glycolytic metabolites are supposed to exist.

Experimental and Results

Test blood

The blood collected from each one *Triakis scyllium* in the groups of instant killing and fatigue mentioned in the previous section, respectively, were used as test material.

Table 3. Distribution of acid soluble phosphorylated compounds and ammonia in the blood obtained from *Triakis scyllium* killed instantly or after fatigued. (mg%)

	Group of instant killing	Group of fatigue
Ammonia N	0.72	1.63
Lactic acid	40	1020
Total phosphate P	40	52
Inorganic phosphate P	3.1	5.0
CP-P	0.4	1.1
Barium insoluble fraction		
Inorganic phosphate P	2.6	2.6
△7P	5.3	3.9
ATP-P	5.5	2.5
ADT-P	3.2	4.5
HDP-P	1.1	0
PGA-P	1.2	0
Barium soluble alcohol insoluble fraction		
Inorganic phosphate P	2.3	4.8
G1P-P	0.7	1.2
G6P-P	12.3	22.5
F6P-P	1.6	0.9
AMP-P	4.4	6.2
TP-P	0	0
PPA-P	0	0
RP-P	0.5	1.7
Barium soluble alcohol soluble fraction		
Total phosphate P	2.9	2.4

Method of analysis

In this test the same method as in the previous one was followed.

Rise and fall of the amounts of acid soluble phosphorylated compounds and the formation of ammonia through fatigue

The analytical results of the amount of acid soluble phosphorylated compounds and ammonia in blood of the groups of instant killing and fatigue are shown in Table 3.

As shown in the table, the amount of lactic acid was very low in the blood of the group of instant killing, while 1,020 mg% lactic acid was contained in the blood of the group of fatigue which was violently shocked in the water tank. It was confirmed also that glycolytic metabolites existed mostly in blood, but neither HDP nor PGA existed at all in the blood of the group of fatigue and F6P was found only little, whereas the other materials were found abundant in the blood of this group.

On the other hand, the level of ATP was high in the blood of the group of instant killing and the levels of ADP and AMP were high in the blood of the other group. The amount of ammonia, in the level of nitrogen, was shown as 0.72 mg% in the blood of the group of instant killing and as 1.63 mg% with of the group of fatigue, which shows that the ammonia content in blood slightly increases by fatigue.

Observations

It was compared that the contents of lactic acid, phosphorylated metabolites and ammonia in the blood of a fatigued *Triakis scyllium* with those in the blood of an instantly killed *Triakis scyllium*. As a result of this comparison the authors confirmed that each of those materials existed in blood, like in the muscle analysed. Lactic acid existing in blood is supposed to be carried to the livers and reformed into glycogen, and it was with much interest to find the existence of not only lactic acid but those metabolites required for the reformation.

Lots of ammonia preserved in muscles are no doubt harmful to the animals. Ammonia, which is one of the final metabolites, stays in urine in its original form in some animals, but very often it changes into such a harmless form as urea or trimethylamine oxide, as excreted[21]. But the ammonia appearing in muscles with glycolysis must not be other than a poison counteracted by some process. From the studies set forth in Parts 1 and 2 it was confirmed that an intense motion increases ammonia in the muscles and blood. However, this dose not mean that glycolysis existed in blood as well as in muscles, but more reasonably it suggests that some of the ammonia in muscles merely went into blood while it being carried to the livers etc. for counteracting poison.

Summary

The lactic acid and ammonia in blood increase in amount by fatigue,

and the main phosphorylated metabolites perceived in muscles exist also in the blood. G6P and the some phosphorylated compounds are preserved though fatigue.

PART 3. CHANGES OF AMOUNTS OF SOME COMPOUNDS IN MUSCLES AND LIVERS THROUGH FATIGUE.

As shown in Table 2, lactic acid created in muscles of the group of instant killing was max. 1,240 mg%, while that of the group of fatigue was max. 1,520 mg%, there being a difference of 280 mg%. This difference is supposed to have resulted from liver glycogen moving into muscles to promote creation of lactic acid.

To make sure of this assumption, the authors examined the consumption of glycogen in muscles and livers, again giving stimulations on two *Triakis scyllium*s bred under the same conditions. The result revealed expectedly that glycogen in livers declines considerably in quantity during the movement of fish.

Moreover, there was made additional researches concerning to the quantities of lactic acid, ammonia and amide contained in muscles and blood, together with the researches of distribution of these materials in the muscles and livers of *Dasyatis akajei*. These researches brought about some new informations, which are given below.

Experimental and Results

Test fish and its treatment

As test fish, a *Dasyatis akajei* (♂ 55 cm. in length) and two *Triakis scyllium*s (♀, 68 and 74 cm. in length each) which were after fishery all bred in Kominato Laboratory Aquarium of Tokyo Univ. of Fisheries were used. First the skate was instantly killed, and from the upper front, upper middle and rear parts of its dorsal body, it was cut off each one slice of test muscles, and from the front, middle and rear parts of its major liver and from the front part of the minor livers, it was took each one slice for analysis. Next a shark (68 cm. in length) was also instantly killed, and the other shark (74 cm. in length) was killed after forcing it swim intensely in the water-tank for 30 minutes in the same way as Part 1. The test bloods of these two sharks were taken for analysis. From their upper front, middle and rear parts and from lower middle parts of body, it was cut off each one slice of muscle. As for livers, each one portion was taken out of the front, middle and rear parts of the two livers, respectively.

Method of analysis

Method of analysis of lactic acid, glycogen and ammonia was the same as Part 1, and amide was calculated from the quantity of ammonia nitrogen increased as a result of hydrolysis by a normal hydrochloric acid at 100°C.

Distribution of glycogen, lactic acid, ammonia and amide in the muscles and livers of *Dasystis akajei*

The results of calculation of the contents of glycogen, lactic acid, ammonia and amide in each part of the muscles and livers of the instantly killed *Dasyatis akajei* are shown in Table 4.

Between the glycogen content in the muscles and that in the livers of *Dasyatis akajei*, there are found considerable differences for various parts of each organ. Muscle glycogen is much in the front muscles and less in the upper muscles, while side muscles retains comparatively much glycogen. On the other hand, liver glycogen content is nearly constant in every part.

Table 4. Distribution of glycogen, lactic acid, ammonia- and amide-nitrogen in the muscle and liver of *Dasyatis akajei*.

	Glycogen mg%	Lactic acid mg%	Ammonia N mg%	Amide N mg%	E_{260}*	E_{248}*
Muscle (upper front)	228	92	3.84	1.95	0.298	0.341
(middle)	115	37	3.75	2.15	0.321	0.325
(upper rear)	99	37	2.81	1.55	0.177	0.213
(side)	218	128	4.55	0.98	0.434	0.588
Liver (front part of major liver)	562	439				
(middle part of major liver)	583	220				
(rear part of major liver)	571	183				
(front part of minor liver)	638	323				

* Test was made on barium soluble alcohol insoluble fractions. The solution used was equivalent to 0.04 g./cc. muscles.

The state of distribution of lactic acid is very interesting. That is to say, lactic acid is rich in the front muscles and especially so in the side muscles, as compared with the middle and rear muscles. This may be that the violent shocks given on the side muscles during the short time from scooping the fish out of the water until the instant murder by clubbing its head caused such a great deal of lactic acid to be created in this part. So interesting is the state of distribution of lactic acid in the livers, too.

The state of distribution of ammonia is very similar to that of lactic acid. Ammonia content is highest in the side muscles, where the optical absorption of barium soluble alcohol insoluble fraction at 260 mμ is also highest. On the contrary, amide nitrogen is lowest in this same part.

Distribution of some compounds in the muscles and livers of *Triakis scyllium* and their changes during fatigue

In Table 5 are shown the results of calculation of glycogen, lactic acid,

ammonia and amide in each part of the muscles, livers and blood of *Triakis scyllium* instantly killed or fatigued.

Table 5. Distribution of glycogen, lactic acid, ammonia and amide nitrogen in the muscles, livers, and bloods of *Triachis scyllium* killed instantly or after fatigued.

		Glycogen mg%	Lactic acid mg%	Ammonia N mg%	Amide N mg%	E_{260}	E_{248}	pH
Group of instant killing	Muscle (dorsal front)	396	268	3.05	1.85	0.203	0.236	6.93
	(dorsal middle)	467	220	3.23	1.94	0.216	0.250	6.89
	(dorsal rear)	527	281	2.88	1.75	0.163	0.218	6.81
	(abdominal middle)	631	250	3.21	1.65	0.193	0.238	6.92
	Liver (front part)	821	98					
	(middle part)	797	140					
	(rear part)	672	24					
	Blood	62*	120	0.63				
Group of fatigue	Muscle (dorsal front)	18.7	1120	9.03	0.56	0.783	1.02	6.11
	(dorsal middle)	21.2	1340	10.2	0.74	0.842	1.23	6.06
	(dorsal rear)	21.5	1180	8.35	0.36	0.765	0.955	6.28
	(abdominal middle)	8.7	1210	9.55	0.75	0.851	1.13	6.16
	Liver (front part)	269	293					
	(rear part)	329	223					
	Blood	33*	538	2.55				

* Blood glucose

Glycogen in the muscles and livers of the group of instant killing is of different densities from part to part, but the livers evidently contain more glycogen as compared with the muscles. However, glycogen declines remarkably thorugh fatigue, that is, it largely disappears in the muscles and lowers to a half or less in the livers. Lactic acid exists in small quantities in both, but exceeds 1,100 mg% in the muscles of the group of fatigue, particularly the largest quantity being found in the middle muscles. And in proportion to the content of lactic acid, pH value of the muscle transfers to the acidic side, showing 6.06 in the upper middle muscles. The lactic acid in blood also rises in quantity through fatigue, quite equally with the result in Part 2. Exceptionally, in this test 120 mg% lactic acid was found in the blood of the group of instant killing.

Ammonia content in the muscle counts 3 mg% or less, with some difference between the parts of the muscles. The amount of this ammonia also increases through fatigue as seen in Part 1, reaching 8 to 10 mg%; especially the upper middle part was found to hold the greatest ammonia. The optical density of barium soluble alcohol insoluble fraction at 248 mμ is slightly higher than that at 260 mμ, and considered from such rapid increases of these optical densities during the

glycolysis, the increase of ammonia may be partly due to the deamination of adenylic acid. On the other hand, the content of amide nitrogen slightly differs from part to part of the muscles, actually showing a decline during the motion.

Observations

In Table 5, it is shown that the upper middle muscles contain the largest quantities of lactic acid and ammonia forming during violent shocks. This seems to suggest that the *Triakis scyllium* swiming in the water moving the middle muscles actively. It has already been shown in Table 4 that the part where the muscles are most actively moved is most distinctive in speed of advancement of glycolysis. *Dasyatis akajei* swim by moving chiefly their side muscles, and the fact that even in the group of instant killing these muscles are more heavily loaded with lactic acid than any other muscles may be attributed to their severe motion during the short time from the scooping to the instant murder.

The decrease of amide induced by creation of ammonia must be paid attention. Fujimaki *et al*[10] count the resolution of amide among the reasons for creation of ammonia, and their assumption has been proved by to be true by our present examination. The creation of ammonia, however, would be little important in quantity.

Putting all these results together, the muscles and bloods of living elasmobranchs contain 3 mg% or less and 0.6 mg% or less ammonia, respectively. For example, when they move from violent shocks at the time of fishery, a remarkable glycolysis takes place followed by creation of ammonia over 10 mg% in the muscles and 2 mg% in the blood. And the longer is continued the motion, the more ammonia is expected to pile up in each organ. Even the muscle of very fresh fish after fishery will likely to hold more or less ammonia in itself depending on the methods of fishing and treatment. It has already been shown that the muscles of such fresh elasmobranchs contain 6 to 24 mg% ammonia[14], but the reason why such a wide range of experimental test values were obtained seems to have to be explained from the difference in degree of the glycolysis that advances in the time of fishery.

Summary

With the test materials taken from the muscles at various parts of the bodies and livers of *Dasyatis akajei* and *Triakis scyllium*, the distributions of glycogen, lactic acid, ammonia and amide were examined. Glycogen in the muscles and livers differs from each other in distribution in different parts, its maximum density ranging 1.5 to 2 times of its minimum values in the muscles, but generally are of high values in the livers with comparatively little difference between various parts of this organ. It was found that lactic acid and ammonia in the muscles of *Dasyatis akajei* are contained somewhat abundantly in the side muscles which are to move most actively.

After letting a *Triakis scyllium* move violentry in a water-tank for

30 minutes, the value of distribution of the above mentioned materials were examined on muscles and livers by using various parts of them as test material. The results, compared with the distribution in the group of instant killing, show marked declines not only in muscle glycogen but in liver glycogen, while lactic acid is preserved in a large quantities in the muscles and bloods. On the contrary, the upper middle muscles were found more remarkable in the rise and fall of these materials than other muscles.

From these tests, the authors came to a conclusion that the muscles behaving actively in motion are also actively in glycolysis and preserve much ammonia in them. This conclusion may provides forms an enough evidence for the fact that much ammonia often exists in the very fresh muscles of elasmobranchs immediately after the fishery.

As to the reason for creation of ammonia, the authors took into account the isolation of ammonia caused by deamination of adenylic acid and resolution of amide, and gave their respective analytical values.

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HAEMATOLOGICAL STUDIES OF THE CULTURE FISHES IN JAPAN
2. SEASONAL VARIATION OF THE BLOOD CONSTITUENTS OF RAINBOW TROUT

Tokuo Sano

(Received Feb. 16, 1960)

Many treatises so far published on the chemical composition of fish blood are concerned exclusively with the constituents happened to be prevalent at a certain time of year or with the demonstrations of the existence of a certain component. For instance, concerning the studies on chemical constituents in blood of salmonoid fish the following reports may be mentioned: Okamura (1936), blood of salmon at the spawning period; Phillips *et al* (1940), glucose of trout blood; Field and coworker (1943), inorganic composition of carp and some of organic components of trout blood; Phillips *et al* (1945), sodium chlolide of trout blood; ditto (1957), the composition of the blood of brown trout for 11 of the inorganic components; ditto (1958), inorganic composition of brown trout blood and organic composition of brook and brown trout blood; Fukuda (1958), blood sugars of Yamame trout (*Onchorynchus milktschitcsh*), rainbow trout and other freshwater fish. Next, a few reports may be cited concerning hematocrit value and blood sedimentation rate for trout and salmon blood. So far as I know no works on hematocrit except the studies by Field *et al* (1943) and Watson and coworker (1956) have bee reoorted. In regard to blood sedimentation rate, I can find only the study of Schumacher *et al* (1956). Those works, however, leave much to be desired, since these authors remain still far from approaching the problem of seasonal variation of the chemical composition of blood, on which this paper presents the results of my investigation of rainbow trout. Investigated items: blood creatinine, blood urea, serum protein, serum albumin and globulin, blood sugar, serum calcium, blood sedimentation rate and hematocrit value. As the present work was carried out with healthy animals severely selected from reared trout, I report the present results only as the values for reared rainbow trout in good health.

Method and Material

The studies were made monthly from January of 1957 to January of 1958, excepting March, September and December. The fish used for experiments were rainbow trout (*Salmo gairdnerii irideus* Gibbons) which hatched in the winter of 1956～1957 in Ayabe Trout Culture Farm* and were accustomed to be fed between 4 and 5 o'clock in the afternoon of the day preceding the experimental use; accordingly, the animals were likely starving for 15～20 hours before the samples of blood were withdrawn. They ranged from 267.0 to 495.0 g in weight and from 23.5 to 29.3 cm in body length.

* In the suburbs of Mishima City, Shizuoka Pref.

Prior to the sampling of blood, the animal caught with net a moment ago was anesthetized by applying an electroshock of A. C. 100v to its head for 5~10 seconds, so that the chemical constituents of blood might not be affected by hyperactivity of muscle on dissecting the belly. After rapid dissection and ascertaining the occurrence of no pathological change in external as well as internal appearance, the samples of blood were withdrawn from animal by means of a caudiac puncture (the needle being inserted into *Bulbus arteriosus*) with a 2cc tuberclin-syringe* equipped with No. 1/3 hypodermic needle. As it proved difficult in preliminary experiment to collect from one individual fish an enough amount of blood for completing a series of analyses, the samples of blood for determining blood sugar, serum protein and hematocrit value were withdrawn from one individual, and the samples of blood for analyses of blood creatinine, blood urea and serum calcium were taken from another individual. Further, for morphological study, a blood film was prepared from each individual.

As field work, determination of total serum protein level, blood sedimentation rate, hematocrit value and a deproteinization of whole blood were carried out for analyses of blood sugar, blood creatinine and blood urea. After blood sedimentation rate was measured by Wintrobe-Landberg's method, hematocrit value was determined by using the Wintrobe's tube. All samples were determined by the same method as that described in a previous report (this Journal, Vol. 43, No. 1).

Result and Discussion

The results are summarized in Table 1. The fiducial ranges for the annual averages of the above items have been obtained by employing t-test on the 5% level. The spots in figures show the average value of five samples taken in each month. As seen in the table or figures, the results obtained show no clear seasonal variations except those for the level of total serum protein, albumin level, globulin level, hematocrit value and blood sedimentation rate. Thus the levels of blood sugar, blood urea, blood creatinine and serum calcium fluctuate within a wide range for each month (Table 1 or Figs. 1, 2, 3, and 5). On the other hand, serum proteins, blood sedimentation rate and hematocrit value present distinct seasonal variations (Figs 4. and 6), the data being not greatly different between different individuals. It is reasoned that such extensive differences as observed in the levels of blood sugar, blood urea and blood creatinine between various months might be caused by the differences in nutritional status or metabolism of each trout at the time of sampling the blood. Five individuals in all the fish treated indicated the level of blood sugar over 210 mg/dl in April, July, October, November, one individual showing 48 mg/dl in May. It is not yet certain whether these values have arisen from hyperglycemia or hypoglycemia. Because of being very unusual they are reserved from the issue.

Fukuda (1958) reported that the blood sugar levels of Yamane trout

* The syringe contained a few miligrams of Heller and Paul anticoagulent, modified by Kato, at the time of drawing the blood for the analyses other than those for serum calcium and serum protein determination.

(*Onchorhynchus milktschitsch*) of 71 and 83 g in weight were 127～131 mg/dl and the levels of rainbow trout (225 and 250 g in weight) 72 and 74 mg/dl. Further, Phillips *et al* (1958) reported that the sugar levels during summer for the brook trout weighing approximately 350 g and the brown trout of approximately 300 g in weight were 70 and 71 mg/dl. Their values, except those for Yamame trout, are approximately a half of the annual average of blood

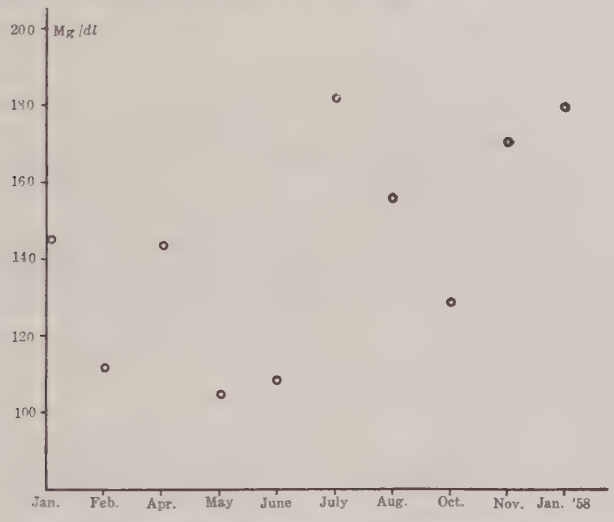

Fig. 1. Showing the changes in the level of blood sugar.

sugar I obtained. The blood sugar levels that Field *et al* (1943) reported for brook trout (stocked in aquarium and of 250 g in average weight) of which greater number were kept abstained from eating, range from 51.4 to 111.0 mg/dl. Accordingly, if their determination were performed with trout fed enough, the levels would perhaps be higher than their values. The reports about the blood creatinine for Pisces are meager. Especially, any informations about the blood creatinine of salmonoid fish cannot be found except the reports of Field *et al* (1943) and Phillips *et al* (1958). Field *et al* reported that the creatinine level of brook trout (250 g in the average weight) in the winter months, November to March, was 0.72 mg/dl on the average. Phillips *et al* reported that the levels of creatinine of brook and brown trout in summer were 0.41 and 0.46 mg/dl. The value of Field *et al* in these results agrees closely with the average value (0.74 mg/dl) of the values I determined in November, January, and February, but the values of Phillips *et al* are about a half of the average value I obtained in summer. Such a difference may be caused by the difference in nutritional status at the time of sampling the blood rather than by the difference in species of fish.

Judging from that the creatinine content of healthy human blood ranges between 0.7 and 1.5 mg/dl, my results may be considered as reasonable in that the blood creatinine content of rainbow trout ranges from 0.4 to 1.5 mg/dl.

Concerning blood urea content, the average value (5.5 mg/dl) of Field *et*

Table 1. Blood constituents of rainbow trout in each month.*

	Jan.	Feb.	Apr.	May	June	July	Aug.	Oct.	Nov.	Jan.'58.	Fiducial range
Blood sugar (mg/dl)	110 168 144.8	103 122 114.0	115 184 143.5	88 112 104.5	84 127 108.5	175 190 182.0	142 183 156.0	125 132 128.8	146 195 170.5	148 180 179.8	$\sigma=31.45$ 139.0 ± 11.18
Blood urea (mg/dl)	3.6 6.5 5.4	4.4 9.0 6.7	1.5 4.1 3.0	1.0 1.4 1.2	2.2 5.1 3.3	1.6 1.9 1.8	4.7 6.9 5.8	3.9 4.8 4.2	3.0 5.1 4.2		$\sigma=1.90$ 3.7 ± 0.62
Blood creatinine (mg/dl)	0.34 0.40 0.37	0.80 0.80	1.48 1.59 1.52	0.49 0.65 0.53	1.52 1.60 1.54	0.73 0.91 0.95	0.93 0.96 0.95	1.18 1.28 1.22	0.94 1.29 1.06		$\sigma=0.383$ 0.99 ± 0.118
Serum calcium (mg/dl)	12.6 16.8 14.7	13.0 18.8 14.7	9.2 13.9 11.6	16.1 16 6 16.4	12.9 16.0 14.4	13.1 17.8 17.4	15.1 19.1 17.5	10.6 14.2 13.6	13.0 18.6 15.1	12.0 14.4 12.8	$\sigma=2.17$ 14.6 ± 2.31
Total serum protein (g/dl)	7.7 8.9 7.5	5.8 8.4 6.8	5.7 7.8 6.4	7.6 9.4 8.30	8.2 9.3 8.8	7.5 8.8 8.2	7.1 8.9 8.1	7.2 8.8 8.2	7.5 9.2 8.4	7.5 8.5 8.2	$\sigma=0.97$ 7.9 ± 0.26
Serum albumin level (g/dl)	7.45 7.01 5.82	4.33 6.28 5.19	4.34 4.93 4.59	6.10 8.45 6.58	5.66 6.25 6.10	5.38 6.09 5.71	5.71 6.42 6.12	5.32 6.51 6.02	5.40 6.85 6.29		$\sigma=0.786$ 5.92 ± 0.242
Serum globulin level (g/dl)	1.39 2.47 1.84	1.28 2.16 1.60	1.21 2.11 1.56	1.50 1.91 1.74	2.17 3.08 2.58	1.91 2.42 2.05	2.14 2.69 2.34	1.83 2.29 2.21	2.10 2.76 2.44		$\sigma=0.468$ 2.20 ± 0.468
Albumin Gloublin ratio	2.26 3.92 3.21	2.86 3.89 3.32	2.34 3.72 3.04	3.32 4.05 3.78	2.04 2.83 2.45	2.22 3.19 2.84	2.13 2.93 2.64	2.84 3.12 2.94	2.33 2.91 2.44		$\sigma=1.652$ 2.98 ± 0.144
Hematocrit (%)	29.8 46.1 38.9	33.3 46.1 38.8	33.6 46.6 39.1	34.9 42.8 38.2	36.6 46.6 40.1	30.0 43.7 36.5	25.2 42.7 33.9	30.0 38.9 34.0	37.1 48.0 40.9		$\sigma=6.06$ 38.6 ± 1.99
Blood sedimentation rate (mm/h)	1.1 1.6 1.4	1.5 1.2 1.3	0.5 1.2 1.0	0.4 0.6 0.5	0.2 0.4 0.3	0.5 0.9 0.6	0.2 0.6 0.5	0.4 0.9 0.5	0.2 0.6 0.4	0.9 1.0 0.9	$\sigma=0.39$ 0.7 ± 0.12

* The figures in upper line give the range of measured values, these in underline the average.

al coincides closely with the average value (5.7) I obtained in winter from November to February. The values (2.8 and 2.9 mg/dl) Phillips *et al* found for brook and brown trout in summer lie intermediate in the range of the values I determined in July and August.

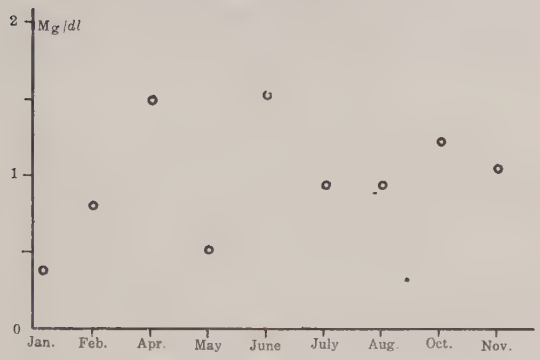

Fig. 2. Showing the changes in the blood creatinine level.

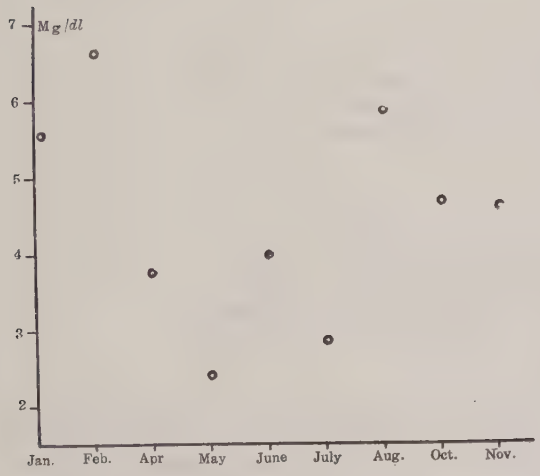

Fig. 3. Showing the change in the level of blood urea.

Regarding the serum protein levels, the globulin is apparently higher in a period from June to November than in that from January to May. On the other hand, such a tendency can not be found for albumin. Accordingly, albumin: globulin ratio tends apparently to low level in a period of January to May and to high level in the time from June to November (Fig. 4). The levels of total serum protein obtained, in July and August, are approximately three times as high as the values reported by Phillips *et al* (1958) for brook and brown trout in summer. And also, the values obtained in November and February are in a range about 2～3 times as high as that of the values Field *et al* (1943) determined for brook trout during November to March. Although there are found some species of fish of a low level of total serum protein in the data that Lepkovsky (1930), Demenier (1935) and Saito (1955) reported for the serum protein of various species of marine fishes, it seems strange and interesting that such large differences are recognized among salmonoid fishes which are similar to one another. However, the results of Field *et al* and Phillips *et al* consist with my result in that A/G ratio is over 2.00.

As Fig. 5 shows, the level of serum calcium tends to high level during summer and to low level from winter to autumn. Namely, it seems that the tendency is associated with the increase and decrease of sunlight or atmospheric temperature. This tendency is similar to the result that Sekine (1929) reported for the calcium content in the whole body of rainbow trout. As the ponds of Ayabe Trout Culture Farm are supplied with spring water of which calcium content and water temperature are almost constant (9.3 mg/l, 15～16°C) all the year round, I

think the level of serum calcium would not be influenced by water. My results are somewhat higher than the values of Field *et al* and Phillips *et al*. This difference may beat tributed to the difference of calcium content of the diet for trout. Namely, in Japan, trout food is made by grinding the whole body of fish (Sardine, common mackerel, sary and etc.), which is to make the predominant animal nutriments for the fish under rearing, with other diet of vegitable source, while, in

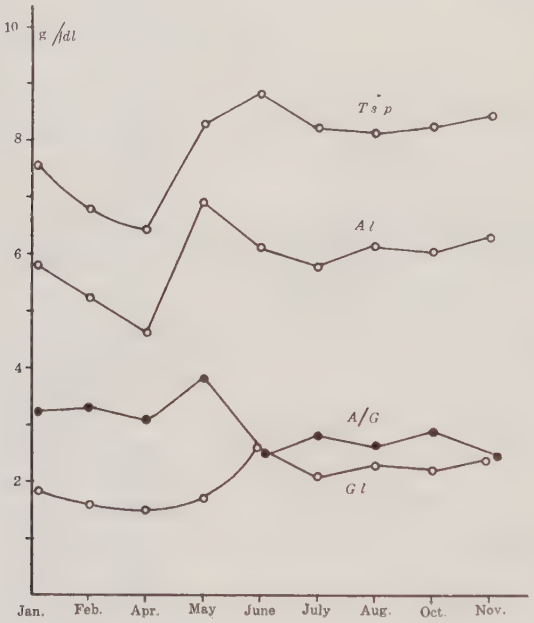

Fig. 4. Showing curves of total serum protein level (Tsp), serum albumin level (Al), A/G ratio, serum globulin level (Gl).

America, feeds for the animal are prepared from mainly beef and pork liver or kidney. So, it is possible that in Japan trout is bred under greater supply of calcium as compared with those of America.

The hematocrit value obtained shows no characteristic maximum and minimum value (Fig. 6). The values of Watson *et al* (1956) for healthy sockeye salmon lie intermediate between the values I obtained. Further the value of Field *et al* is lower by over 5~10 mg than my value for the same season, on an average. Concerning the blood sedimentation rate, there are differences between the values (1.75~1.88 mm/h, mean value of Katz) obtained in October by Hoshina (1953) for rainbow trout weighing 1650 to 1900 g or those reported by Schumacher (1956) and the values I obtained. This discordance may have arisen certainly from the difference of method: Westergren and Wintrobe-Landsberg.

In spite of measurements under 15 to 22°C, the results in summer months tend to low values (Fig. 6). This question has not been solved from the data concerning serum protein, hematcorit value and etc.

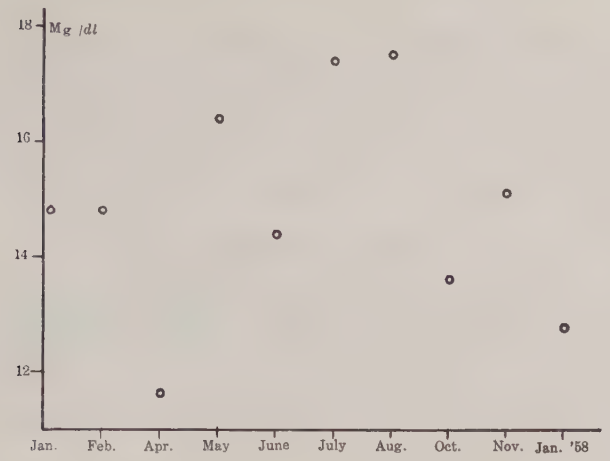

Fig. 5. Showing the changes in the level of serum calcium.

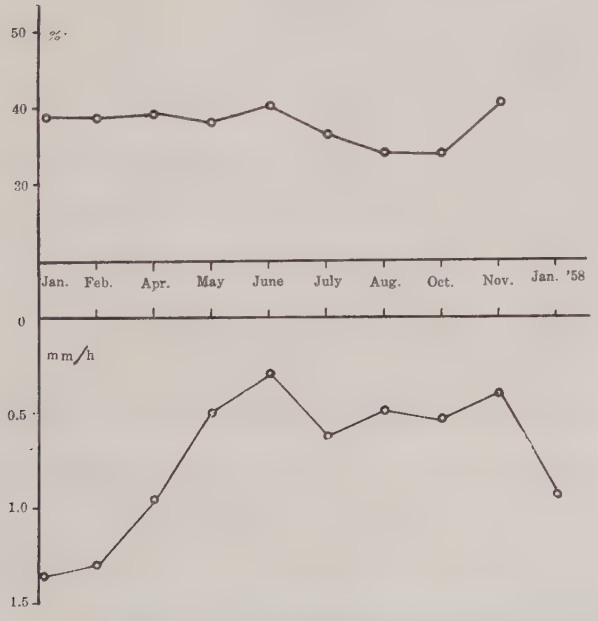

Fig. 6. Showing curves of hematocrit values (upper curve) and erythrocyte sedimentation rate (lower curve).

My intention is to enter in future into detailed studies about the change in some chemical components, especially the characteristics of serum proteins, hematocrit value and blood sedimentation rate possible to change with the growth of trout.

Summary

The following matters were determined for reared rainbow trout: blood sugar, blood urea, blood creatinine, total serum protein, serum albumin, serum

globulin, serum calcium, hematocrit value, and blood sedimentation rate. The levels of blood sugar, serum calcium, blood urea and creatinine were found to fluctuate generally in wide ranges depending not only on the month of measurement but on the fish individuals examined, while the levels of serum proteins, blood sedimentation rate and hematocrit value varied scarcely to any significant extents with the shift of the seasons.

Acknowledgment

I wish to thank Mr. H. Ayabe of the master of Ayabe Trout Culture Farm for his kindly supplying valuable large trout demanded for the study. I wish to my sincere appreciation to Prof. T. Hoshina of the Tokyo University of Fisheries for his permitting to cite his unpublished data concerning blood sedimentation rate. I also thank Prof. Dr. H. Hirano for critically reading this manuscript.

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HAEMATOLOGICAL STUDIES OF THE CULTURE FISHES IN JAPAN 3. CHANGES IN BLOOD CONSTITUENTS WITH GROWTH OF RAINBOW TROUT

Tokuo Sano

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Hitherto, the health-management of fish in culture farms resorted only to the external finding of fish. In general, when a randamly selected small number of fish are observed in extremely bad health, also the rest of fish may well be suspected to be on the threshold of a desperate state of affairs. Actually, a considerable high mortalities of rainbow trout, which might be caused by a bacterial or unknown disease, have been experienced in various trout culture farms in Japan. In such a ones except special ones, before the occurrence of mortality, chemical compositions of blood are supposed to be disturbed in metabolism. Then, if it is able to previously find the abnormalities of chemical constituents by regular diagnosis, and that by the routine analysis for clinical purpose as used in medical science, it will be able to take some adequate measure against the disease so that the mortality of fish may not become serious.

I found that the values of hematocrit and the levels of total serum protein of trout affected by unknown agent were lower than those in good health, and especially that the determination of serum protein made an indispensable adjunct in judging whether the fish is healthy or diseased. However, the informations concerning the standard values of serum protein levels and hematocrit for various sizes of salmonoid or other fish in the condition of health are lacking, not to speak of the reports dealing with the problem as to whether these values do or do not change with growth. Here, I report the results of my measurements of serum protein level and hematocrit value obtained monthly, following up the growth of rainbow trout.

The studies for two year old trout were carried out taking account of the change in blood components accompanying the development of their gonads.

Method and Material

Measurements were made monthly from April to December, in both years of 1958 and 1959. The fish used for sampling the blood were rainbow trout (*Salmo gairdnerii irideus* Gibbons) severely selected as healthy from those which were kept reared after hatching in Ayabe Trout Culture Farm*. For the study in 1958, the trout which hatched in January, March of 1958 and in January of 1957 and were found to have grown up to 3.3 g (3 months old), 0.4 g (1 month old) and 309.0 g (14 months old) in average weight, respectively, at beginning of study, April, were used. And also, the trout which hatched in February of 1958

* In suburbs of Mishima-City, Shizuoka-Pref. The ponds are supllied mainly with spring water whose temperature ranges from 15 to 16°C throughout the year.

and were found to have grown up to 269.6 g (13 months old) in average weight in April of 1959, were used for the study in 1959. In December, these 2 year old trout (especially, the trout used in December of 1959) were found to have matured so fully that eggs and sperm were expressed from the animals on gently pressing their belly by hand. Concerning 1-and 2-yearling trout, three individuals for each sex were treated every month.

For sampling the blood from small trout (down to 5 month old fingerling), the blood dropping on cutting off the head or caudal peduncle was collected. It was necessary to pool the blood from individual fish up to a total volume over 0.5 cc. The sampling of blood from 1-and 2-yearling was made by the same method as described in my previous report.

First, the blood for hematocrit test was withdrawn, next, the blood for separating the serum was collected directly into a test tube from *Bulbus arteriosus*. The separation of blood serum completed by centrifusing after allowing the blood to clot in the tube kept in a slanting position at room temperature or in a refrigerator.

Hematocrit value was measured by means of Wintrob's tube (submitted to in revolution for 30 minutes at the rate of 3000 r.p.m.). The level of total serum protein was determined by Hitachi hand protein refractometer and 4 fractions in serum, viz., albumin, alpha-globulin, beta-globulin and gamma-globulin were determined according to Yoshikawa and Saito's modification of Kingsley'method.

Result

The results are summarized in Tables 1~5. The spot in figure for 1-and 2-yearling shows the average value of six samples taken in each month, and also, the spot in figure for fingerling trout shows an analytical value for blood sample of pooled from 5 to 15 individuals in each month. The level of total serum protein in fingerlings is higher for large fingerling trout than for small one and these proteins increase gradually with the growth of fingerling trout (Table 1 and Fig. 1).

Table 1. Serum protein level changing with the growth of fingerling trout

		Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Small fingerling trout	B.W(g)	0.37	0.84	1.90	2.5	4.5	8.1	9.5	15.9	18.2	25.6
	B.L (cm)	3.5	3.9	4.9	5.1	6.4	8.0	8.8	9.6	10.2	11.1
	T.S.P** (g/dl)	3.1	3.4	2.9	4.1	4.5	4.8	4.7	5.0	5.0	5.6
Large fingerling trout	B.W (g)	3.3	5.7	17.4	30.3	63.2	109.8	178.2	420.0*		
	B.L(cm)	5.7	6.7	9.4	12.4	13.7	17.8	20.4	26.8		
	T.S.P** (g/dl)	4.2	4.2	5.0	5.2	5.9	5.8	6.2	7.2		

* The largest fingerling unexpectedly encountered.

** The level of total serum protein.

The tendencies of increase are seen for all the fractions examined but for gamma-globulin (Fig. 2).

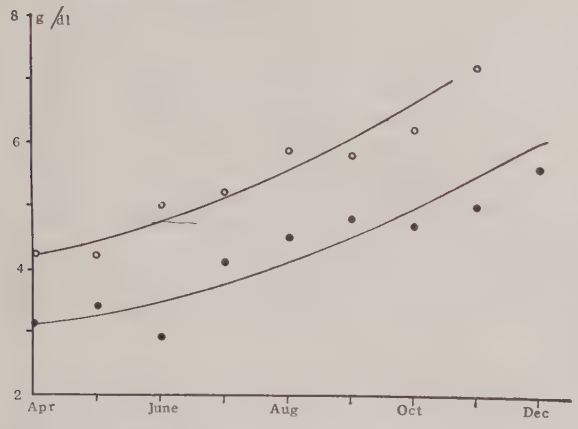

Fig. 1. Curves for total serum protein level changing with the growth of fingerling trout. Upper curve, for small fingerling; lower curve, for large fingerling.

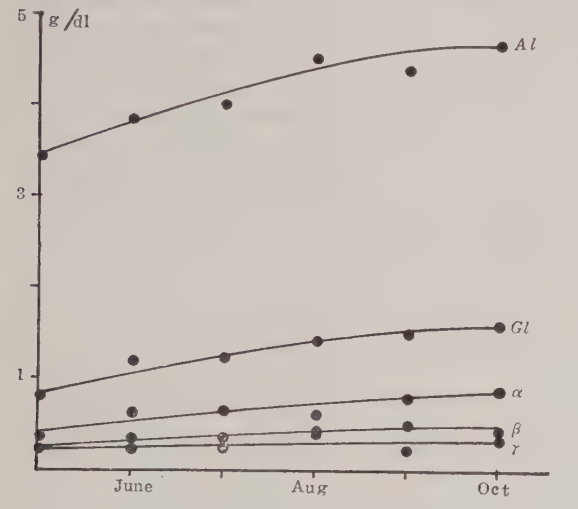

Fig. 2. Changes of each fraction in serum protein with the growth of fingerling trout. Al, albumin; Gl, globulin; α, alpha-globulin; β, beta-globulin; γ, gamma-globulin.

The levels of total serum protein of 1-and 2-yearling trout are higher in female than in male, especially for the months after September. The annual variations with growth, however, represent a tendency equally prevalent in both sexes. Such variations run parallel with the increase and decrease in albumin fraction, excepting the case of globulin fractions in female. Namely, after August the globulin in female rises with a considerable rate (Table 3 and Fig. 3). Explaining particularly the changes in globulin fraction, alpha-globulin level in female once decreases and once increases ranging between 0.60 and 1.28 g/dl from April to July, and subsequently rises up to an extremity of maximum level (3.31 g/dl) in December (Fig. 4). However, the change in male is not so remarkable as in female, viz., alpha-globulin level after August scarcely exceeds the yearly average level (Fig. 4). Regarding beta-globulin, the level for female increases from August, reaching to a maximum value (1.25 g/dl) in September and subsequently decreasing in a distinct manner (Fig. 5). On the contrary, no peculiar change can be found for male (Fig. 5). Gamma-globulin level forms plateau throughout a period from April to September for both sexes, ranging from 0.34 to 0.51 g/dl in female and from 0.32 to 0.59 g/dl in male, but from autumn to winter a distinct fall is recognized for each sex (Fig. 5).

Hematocrit value of male increases gradually from April (37.2%), reaching to an average peak value (45.8%) in September; but in female it reaches to 38.0% in September, ranging from 33.2% to 38.0%. Subsequently, the values for both

Table 2. The distribution of serum proteins changing with the growth of large fingerling trout.

	May	Jun.	Jul.	Aug.	Sept.	Oct.
Albumin (g/dl)	3.42	3.83	3.99	4.49	4.31	4.62
Globulin (g/dl)	0.78	1.17	1.21	1.41	1.49	1.56
Alpha-globulin	0.33	0.62	0.64	0.59	0.79	0.87
Beta-globulin	0.22	0.33	0.34	0.42	0.49	0.42
Gamma-globulin	0.22	0.22	0.23	0.40	0.21	0.29
A/G ratio*	4.405	3.285	3.283	3.176	2.887	2.927

* The ratio of albumin to globulin is given as "A/G ratio."

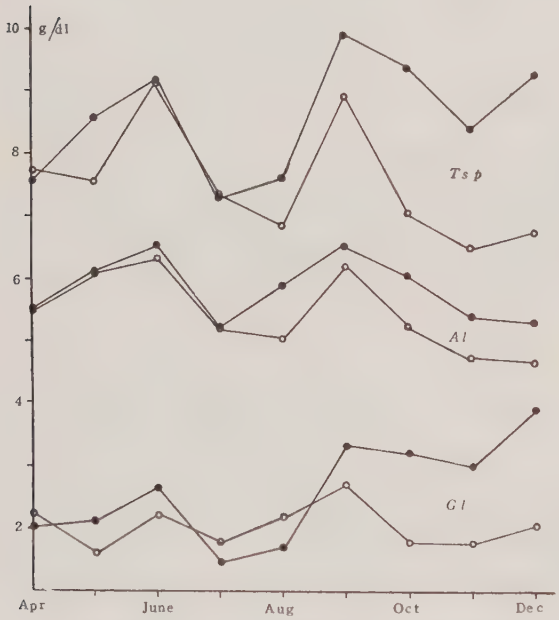

Fig. 3. Showing the changes in the level of total serum protein (Tsp), serum albumin level (Al) and serum globulin level (GL) accompanying the growth and maturation of 1-and 2-yearling trout. Closed circles, female; open circles, males.

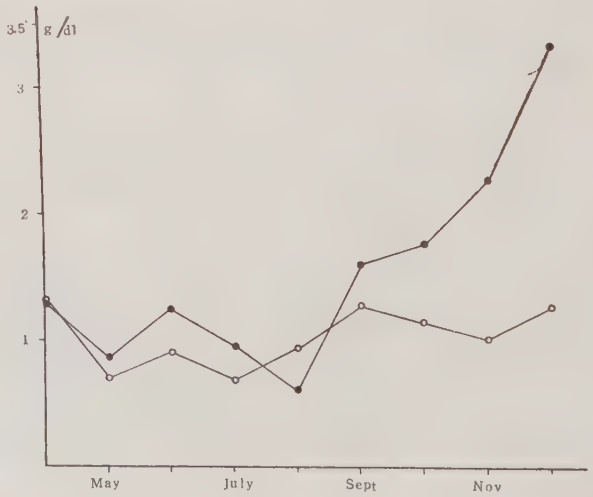

Fig. 4. Changes in alpha-globulins of female (closed circles) and male (open circles).

Table 3. Changes in the serum proteins with the growth of female trout

		Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Fiducial range at 95%
Albumin (g/dl	Range	4.69 6.36	4.83 6.82	5.63 7.24	4.95 5.48	4.48 7.73	5.97 7.69	4.63 7.37	4.55 5.93	4.89 5.57	$\sigma=0.479$
	Average	5.52	6.10	6.50	5.22	5.85	6.49	6.02	5.35	5.26	5.88 ± 0.391
Alpha-globulin (g/dl)	Range	0.79 1.66	0.71 1.27	1.00 1.61	0.56 1.34	0.08 0.97	1.04 2.19	1.23 2.45	1,06 2.93	1.96 5.98	$\sigma=0.642$
	Average	1.28	0.86	1.24	0.95	0.60	1.58	1.75	2.26	3.31	1.60 ± 0.642
Beta-globulin (g/d.)	Range	0.28 0.66	0.50 1.14	0.59 1.35	0.54 0.74	0.25 1.33	0.39 2.00	0.12 2.28	0.14 1.01	0.03 0.53	$\sigma=0.272$
	Average	0.42	0.81	1.00	0.64	0.79	1.25	1.10	0.70	0.41	0.81 ± 0.222
Gamma-globulin (g/d.)	Range	0.15 0.64	0.29 0.50	0.21 0.95	0.26 0.63	0.20 0.59	0.17 0.83	0.00 1.02	0.12 0.39	0.00 1.16	$\sigma=0.150$
	Average	0.34	0.44	0.40	0.45	0.41	0.51	0.39	0.03	0.27	0.44 ± 0.122
Serum protein (g/dl)	Range	6.0 9.0	6.4 9.6	8.0 10.6	7.1 7.4	6.1 8.9	8.9 11.5	6.3 12.5	6.4 9.6	7.0 12.3	$\sigma=1.55$
	Average	7.6	8.5	9.1	7.3	7.6	9.8	9.3	8.3	9.2	8.6 ± 0.45
A/G ratio	Range	2.306 3.575	2.541 3.080	2.140 2.713	2.309 2.854	1.922 7.065	1.612 2.556	1.388 2.979	1.555 2.455	0.773 2.295	$\sigma=1.2385$
	Average	2.872	2.912	2.488	2.580	4.264	1.199	2.140	1.853	1.643	2.530 ± 0.3732
Body weight (g)	Range	220 350	270 360	340 480	680 720	538 780	610 815	640 923	789 1200	880 1307	$\sigma=267.39$
	Average	267.5	323.4	430.0	695.0	638.2	680.5	748.6	966.4	1096.3	646.9 ± 141.72
Body length (cm)	Range	22.6 26.6	24.3 26.7	24.6 30.0	32.0 32.5	27.8 33.3	29.1 35.4	29.0 36.0	31.4 38.2	36.9 39.8	$\sigma=5.05$
	Average	22.2	25.7	27.6	32.3	31.1	32.0	34.0	34.8	37.5	32.5 ± 2.68
Gonad weight (g)	Range		0.6 1.4	1.3 3.3	2.8 4.0	9.0 17.6	16.5 46.0	33.0 70.0	56.4 104.9	155.0 232.0	
	Average		1.0	2.4	3.4	10.7	25.8	46.8	85.1	176.4	

Table 4. Changes in the serum proteins with the growth of male trout.

		Apr.	May.	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Fiducial range at 95%
Albumin (g/dl)	Range	4.79 6.19	4.79 6.24	5.37 6.99	4.80 5.94	4.20 6.06	5.27 8.28	4.63 6.26	3.98 5.18	3.76 6.48	$\sigma=0.552$
	Average	5.46	5.59	6.30	5.37	5.03	6.17	5.20	4.68	4.62	5.40 ± 0.450
Alpha-globulin (g/dl)	Range	0.89 1.84	0.55 0.84	0.90 1.25	0.36 1.00	0.64 1.38	0.96 1.76	1.06 1.19	0.87 1.29	0.71 1.79	$\sigma=0.227$
	Average	1.32	0.72	0.90	0.68	0.94	1.27	1.13	1.04	1.28	1.06 ± 0.185
Beta-globulin (g/dl)	Range	0.35 0.57	0.36 0.74	0.64 1.41	0.50 0.58	0.29 1.44	0.49 1.15	0.34 0.48	0.35 0.82	0.12 0.57	$\sigma=0.198$
	Average	0.47	0.56	0.96	0.54	0.68	0.83	0.39	0.55	0.29	0.59 ± 0.161
Gamma-globulin (g/dl)	Range	0.38 0.51	0.20 0.44	0.00 0.78	0.58 0.59	0.27 1.12	0.17 1.29	0.06 0.80	0.00 0.35	0.11 0.49	$\sigma=0.144$
	Average	0.45	0.32	0.35	0.59	0.53	0.59	0.27	0.21	0.16	0.39 ± 0.006
Serum protein (g/dl)	Range	6.5 9.1	6.0 9.0	7.0 11.0	6.2 8.1	5.6 7.1	7.5 12.0	6.4 8.5	6.0 7.3	5.0 9.2	$\sigma=1.41$
	Average	7.7	7.5	9.1	7.3	6.5	8.9	7.00	6.4	6.7	7.5 ± 0.46
A/G ratio	Range	2.165 2.802	3.354 3.715	1.740 3.382	2.741 3.337	1.540 3.500	1.896 3.006	2.615 3.170	2.308 3.191	2.378 3.043	$\sigma=0.4804$
	Average	2.487	3.490	2.782	3.090	2.655	2.370	2.928	2.702	2.732	2.764 ± 0.1555
Body weight (g)	Range	220 410	300 340	330 520	545 740	499 740	497 860	451 710	820 1240	604 1092	$\sigma=226.28$
	Average	307.8	313.3	457.5	700.0	584.1	676.0	597.0	965.9	873.1	602.9 ± 119.92
Body length (cm)	Range	22.3 27.4	23.5 26.1	24.8 29.5	31.0 34.0	28.0 32.4	29.5 34.6	29.0 34.1	35.6 41.0	35.0 37.9	$\sigma=4.22$
	Average	25.5	24.9	27.7	32.9	31.2	32.4	31.3	36.2	6.0 35.9	30.6 ± 2.24
Gonad weight (g)	Range		0.7 1.0	0.4 1.2	1.5 4.3	6.0 22.0	6.4 57.4	19.0 40.5	28.5 68.0	37.0 67.5	
	Average		0.9	0.9	2.9	11.7	23.0	27.8	45.3	48.0	

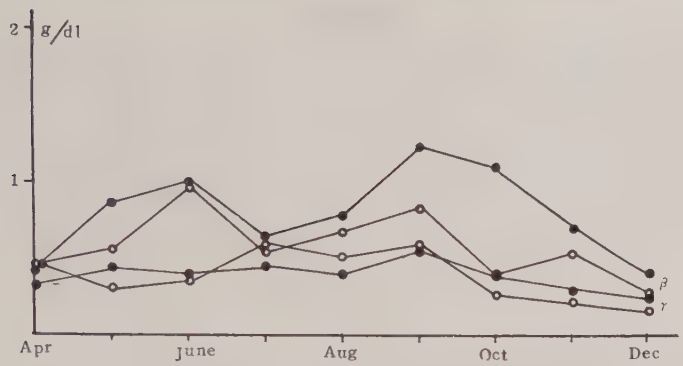

Fig. 5. Curves for the changes in beta-and gamma-globulins shown for both sexes. Closed circles, female; open circles, male.

Table 5. Changes of adult trout in hematocrit value.

		Apr.	May.	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Fiducial range at 95%
Female	Range	21.9	25.0	25.0	39.3	31.9	33.6	15.0	20.1	17.0	
		45.0	49.2	45.2	40.6	35.0	43.8	36.3	40.0	23.8	$\sigma=8.66$
	Average	36.6	36.6	33.2	40.0	34.3	38.0	28.7	30.3	19.4	33.0 ± 2.66
Male	Range	28.0	25.0	30.0	36.5	31.8	35.0	17.5	16.3	10.0	
		48.0	50.0	51.5	42.5	40.6	55.0	43.4	40.6	37.5	$\sigma=10.15$
	Average	37.2	35.0	39.8	39.5	37.5	45.6	32.6	29.9	21.9	36.0 ± 3.29

sexes fall remarkably, reaching to 21.9% (male) and 19.4% (female) in December. And also, the values for male are always higher than those for female (Table 5 and Fig. 6).

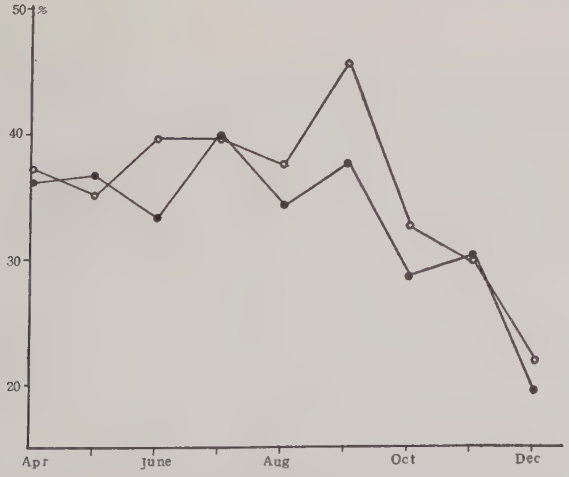

Fig. 6. Changes in hematocrit value of female (closed circles) and male (open circles).

Discussion

It is interesting that the level of total serum protein in trout blood rises with growth, globulin fraction changing more markedly than albumin fraction. Namely, regarding fingerling trout, as seen in Figs. 2 and 7, globulin fraction continues a gradual increase till September, and also, alpha-and beta-globulins. On the other hand, in October, the average body weight reached to a value of 54 times of the first weight (Fig. 7). From such matters, it may be inferred that alpha-and beta-globulins are intimately related to the growth of fingerling trout.

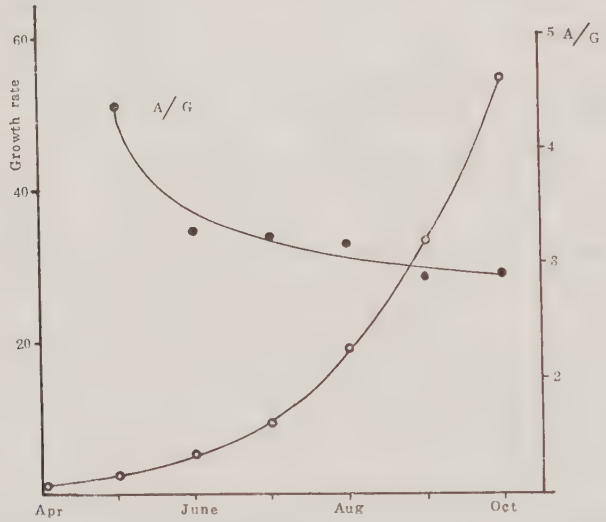

Fig. 7. Relative growth curve and A/G ratio. Growth rate (left ordinate) is shown as the ratio of the body weight in each month to the weight in April.

It is of immunological interest that gamma-globulin level of fingerling trout is almost always constant and remains lower than that of adult trout. The serum protein level of two largest fingerlings, 430 g and 410 g in weight and 27.0 cm and 26.6 cm in lenght, which were obtained unexpectedly on October 22 of 1958, were 7.5 and 7.4 g/dl, respectively. Thus, it becomes more and more certain that the level of serum protein tends to change according to the difference in degree of growth, even among trout individuals of the same age (Table 1). This point agrees with the opinion of Saito (1957) that total serum protein tends to higher level in adult shark than in immature one.

From the comparison between the changes of serum protein level for fingerling trout and adult trout, interesting fluctuations, which never occurred in fingerlings, are recognized to take place during June to August and September in adult trout serum. On the basis of Figs. 3, 4, 5, and 8, it is possible to correlate these fluctuations with the development and maturation of gonad. Thus, the reduction of total serum protein observed from June to July or August may be due to formation of early reproductive organ and the reduction after

September may be caused by full maturation of gonad. Explaining in detail the change in serum protein level, albumin fraction which falls in July or August, recovers in September and again decreases apparently with total serum protein, in both sexes, at variance with the development of gonad. On the contrary, globulin fraction in female rises remarkably with the development of gonad from August, though the tendency is not distinct in male. The results is that A/G

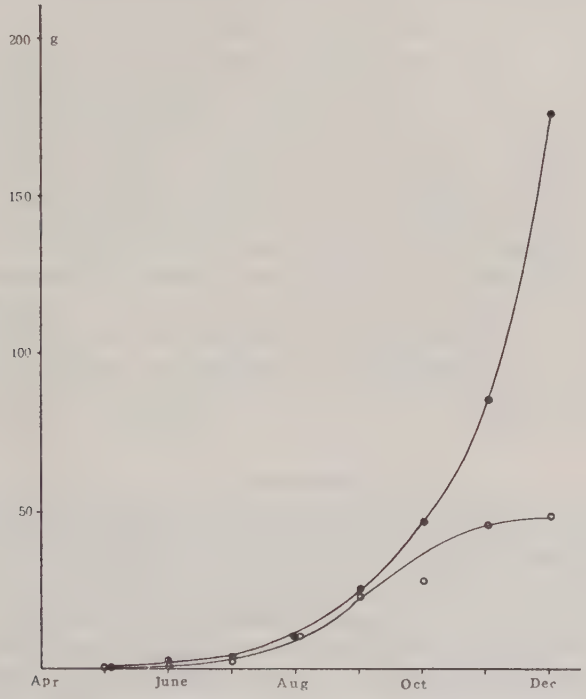

Fig. 8. Maturation curves of gonads shown in weight. Closed circles, female; open circles, male.

ratio for male becomes higher than that of female from September (Tables 3 and 4). Such a tendency in alpha-and beta-globulins is characteristic of female. Namely, alpha-globulin level rises remarkably, reaching in December a value about three times of the monthly average in the period from April to August. Beta-globulin rises from August and reaches the average peak value (1.25 g/dl) in September, decreasing subsequently. In male, on the other hand, alpha-globulin tends to increases in some degrees from July and beta-globulin rises faintly in August and September, subsequently reducing

Gamma-globlin shows for both sexes a similar tendency without considerable changes, beginning to reduce from September to October (Fig. 5). However, it is doubt, if the reduction is associated with the maturation of gonad. And, nonexistence of sexual difference in gamma-globulin level may immunologically be a matter of course. This particular study is reserved for future.

Shinozaki *et al* (1951) reported that the level of total serum protein fell during human pregnancy. Von Studnitz (1956) reported for the serum protein

during human pregnancy as follows: The concentrations of the total serum protein and serum albumin decrease significantly during pregnancy. The alpha- and beta$_1$-globulins increase significantly, but no significant changes occur in the gamma and beta$_2$ globulins.

Larson and Kendall (1957) obtained the results that the levels of serum proteins in blood of bovine started increasing to reach a maximum in about 4 weeks before parturition and then started decreasing to reach a minimum at parturition. Thus, it is very interesting that the changes in blood serum protein levels associated with the development of the ovary of trout occur similarly to the changes in the serum proteins during mammalian pregnancy.

As Pora (1936) reported, the level of total serum protein is higher in females than in males in the present result, too. Besides, female is on a higher level than male in respect of alpha-and beta-globulins.

The reductions of hematocrits associated with the development of gonad in both sexes are essentially similar to the result Kaplan and Crouse (1956) reported about the hematocrits of frog (*Rana pipiens*): The decrease of pcv$_s$ (= hematocrit value) in spawning season was especially great in females having laid eggs. Except for the spawning season, male pcv$_s$ were significantly higher than those of females. Such reductions of hematocrits may be caused by similar phenomenon to the anemia which occurs in mammalian pregnancy.

Summary

The changes in serum protein levels and hematocrit value with the growth of rainbow trout were studied from April to December of 1959 and 1958. The results are as follows: In the case of fingerling trout, the levels of total serum protein, alpha-and beta-globulins rise smoothly with the growth. In the case of adult trout, the levels of the total serum protein, albumin and beta-globulin increase till June by significant measures with the growth. Subsequently, the changes in serum protein levels occur with the development of gonads in both sexes. Namely, the low levels are attained in July or August, a maximum being reached in September with subsequent beginning to decrease again from October. Female alpha-globulin increases from August apparently at a greater rate with the development of ovary as compared with male alpha-globulin. Female beta-globulin increases apparently till it reaches a maximum in September, decreasing again. No such significant change occurs in male beta-globulin as that in female beta-globulin.

The level of gamma-globulin in fingerling trout continues to be almost constant, but the levels of adult (1-and 2-yearling) trout begin to reduce in both sexes from September. Hematocrit value falls remarkably with the development of gonads in the both sexes.

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HAEMATOLOGICAL STUDIES OF THE CULTURE FISHES IN JAPAN
4. METHOD FOR REPEATED DRAWING OF BLOOD FROM CUVIERIAN DUCT

Tokuo Sano

(Received Feb. 15, 1960)

Repeated drawing of blood from one and the same individual of fish without dealing a fatal effect makes a knotty problem often met with in certain sections of fish investigation such as physiology, biochemistry, pathology, etc. of fish. However, in so far as fishes are concerned, reports dealing with the method of blood drawing are extremely scanty. In process of the preparation of this paper, Schiffman reported a method of repeated sampling of trout blood. However, as compared with the Schiffman's method, my method presented in this paper is thought to be more convenient.

Cuvierian ducts are externally visible in intact trout. Namely, Cuvierian duct exists showing the blood colour under the epidermis which covers a clavicle and the supporting tissue. Accordingly, the position of the duct can be sought by turning up the fourth gill arch.

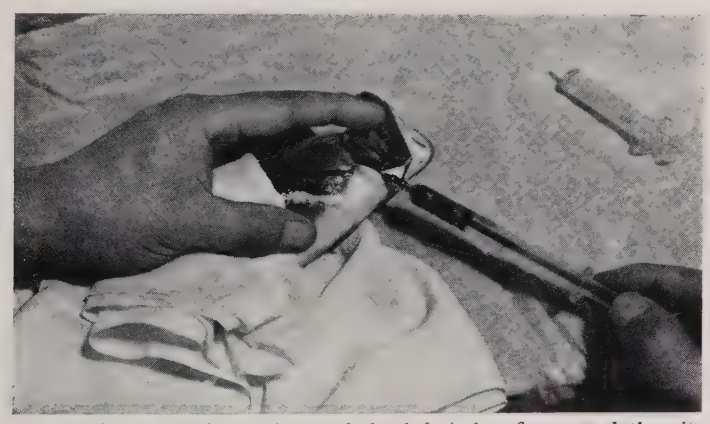

Figure illustrates the position of the left index finger and the site of puncture. For taking a photograph, operculum is supported upright rather exaggeratedly.

Method is that the blood can be withdrawn from trout by inserting into Cuvierian duct a 2cc-tuberclin syringe with a No. 1/4 (for small trout) or No. 1/3 (for large trout) hyperdermic needle. As the above figure illustrates, a fish is placed on a plane and fixed by pressing the left hand on the body. Next, the operculum and all gill arches are turned up with pincette in the right hand. Next, the left index finger supports up all gill arches and operculum, and the right hand manages a syringe. The needle should be inserted nearly in parallel

with the clavicle. As soon as the necessary volume of blood is obtained, the fish is transfered for rest in calm flow enriched with oxygen so as to recuperate from hemorrhagic shock.

The blood of rainbow trout ranging from 50 to 900 g in weight were withdrawn by means of the present method. The result are summarized in table.

No.	Body weight (g)	Blood volume withdrawn(cc)	Remark
1	50	0.2	Unanimated after sampling, but servived.
2	100	1.0	After drawing, died within three hours with opercular tremors and loss of equilibrium.
3	110	0.5	Swan vigorlessly near the water surface for half a day, but recuperated afterwards.
4	120	0.75	Somewhat weakened after sampling. While the death was not expected, died 24 hours after.
5	200	1.0	Showed temporary loss of equilibrium. Survived.
6	600	2.0	Survived. After one month the same volume was drawn again in safety.
7	900	4.0	Survived. After two months the same volume was sampled again in safety.

The result was that it is possible to draw the blood volume of 5/1000 of body weight. All the fish examined tended to the increase of respiratory rate, temporary loss of equilibrium, opercular spasm, temporary loss of vigour and decoloration of skin. All survived are breeding, now, after the lapse of three to six months, in Ayabe Trout Culture Farm. The results obtained and the reaction to blood loss agree with Schiffman's report. Schiffman reported that the blood sample can be taken in a matter of 15 to 30 seconds by an experienced technician from the dorsal aorta in the roof of the mouth of the trout anesthetized, but by means of my method the blood can be withdrawn within similar seconds without anesthetizing a trout. Further, advantages of the present method are as follows: When draws of blood from one Cuvierian duct was unsuccessful, the blood can be taken from the other duct; the blood volume of 5~7.5/1000 of body weight can be drawn from the fish having been dead for some hours.

The present method will be able to be applied to the other various species of fish except eel and conger.

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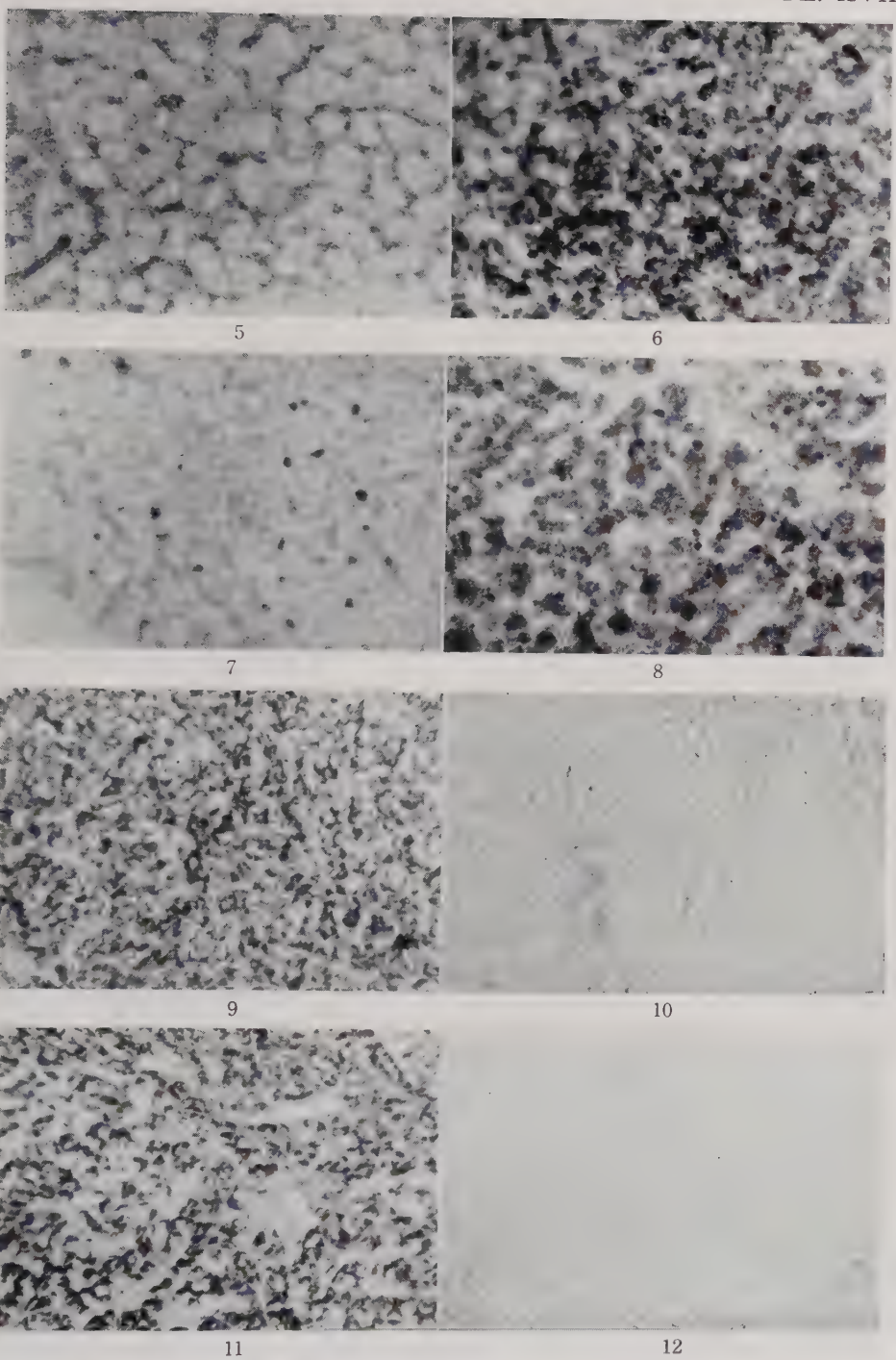
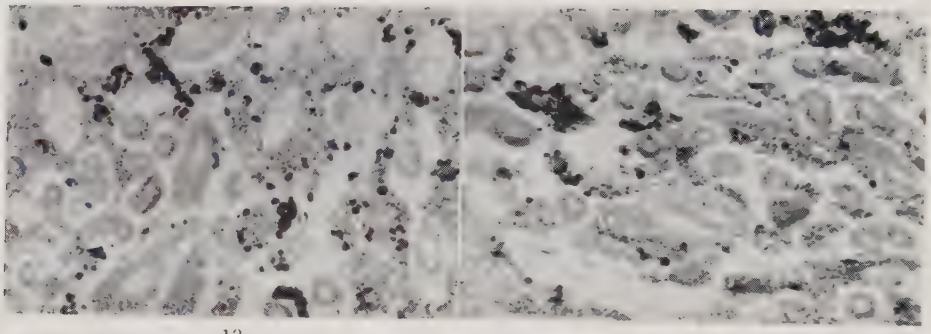


Figs. 5 to 8. Lipid in liver (Acetic Carbol-Sudan III. single stain).
Fig. 5. Group I.
Fig. 6. Group V.
Fig. 7. Group VI.
Fig. 8. Group VII.
Figs. 9 to 12. Glycogen in liver (Bauer-Feulgen's reagent, single stain).
Fig. 9 Group I.
Fig. 10. Group V.
Fig. 11. Group VI.
Fig. 12. Group VII.



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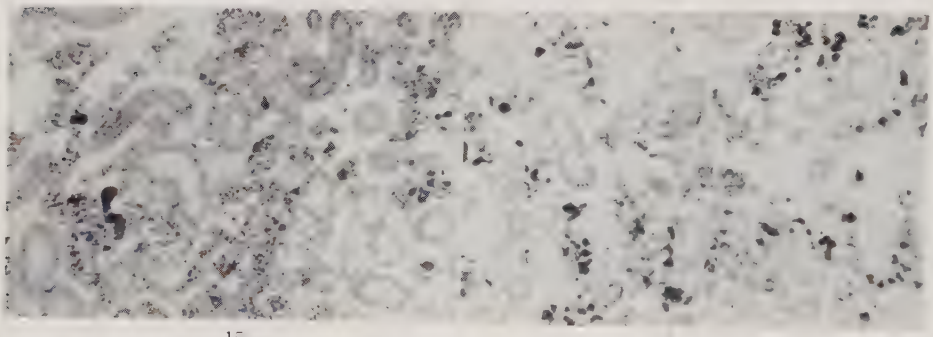

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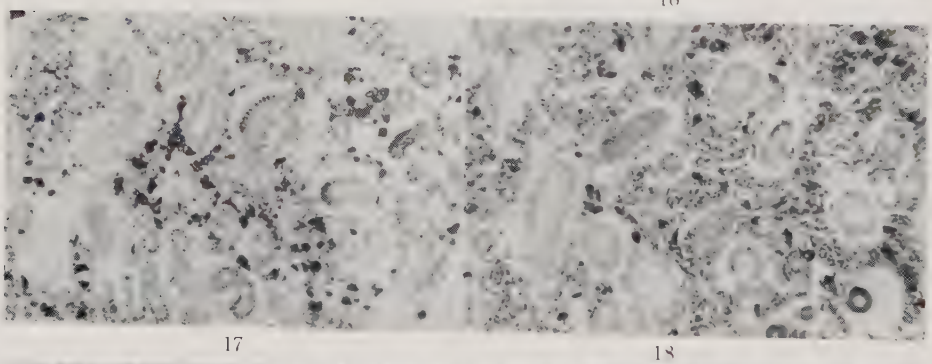

17 18

19 20

Figs. 13 to 18. Kidney (Ehrich's Hematoxylin-Eosin, double stain).
Figs. 13 and 14. Group I.
Figs. 15 and 16. Group V.
Figs. 17 and 18. Group VII.
Figs. 19 and 20. Liver (Ehrich's Hematoxylin-Eosin, double stain).
Fig. 19. Group I.
Fig. 20. Group VII. Gap among tissue is supposed to be a trace of lipid which was extracted with solvent.

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NOMOGRAMS OF LOGISTIC CURVE

Nin KUMASAWA

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§ 1. Introduction

The problem to find the value of parameters of a logistic curve when the number of individuals for equal time interval are given has been contributed by many authors with many methods, one of which is turning into account nomograms using ratios of the number as arguments.[1),2)]

Nomograms are also utilized in this note, however, preliminary ratio-calculation for arguments is avoided.

§ 2. Construction of the nomograms

The equation of a logistic curve is given by

$$N=L/\{1+\exp(a-bt)\} \tag{1}$$

here t represents time and N the number of individuals and L,a and b are constants whose value are to be found.

Let given value of N for time $t=0,1,2$ are N_0, N_1, N_2 respectively, then we can obtain

$$L=\left(\frac{1}{N_0}+\frac{1}{N_2}-\frac{2}{N_1}\right)\Big/\left(\frac{1}{N_0N_2}-\frac{2}{N_1^2}\right) \tag{2}$$

and

$$N_0N_2<N_1^2$$

is necessary for these N's lie on a logistic curve (1).

When equation (2) is rewritten as

$$L-N_2=\frac{(N_2-N_1)^2}{n-N_2}\,,\quad n=\frac{N_1^2}{N_0}$$

we can obtain the value of L making use of a nomogram of equation

$$z=\frac{y^2}{x} \qquad \text{(CHART I)} \tag{3}$$

two times, that is

1 Find $z=n$, when $x=N_0$, $y=N_1$

2 Find $z=L_0$ when $x=n-N_2$, $y=N_2-N_1$

then the value of L is $L=L_0+N_2$.

To find the value of a, b after obtained the value of L we construct a nomogram

$$L=N(1+e^{\tau})\,,\qquad \tau=a-bt \qquad \text{(CHART II)} \tag{4}$$

Let the value of τ colinear with L and N_0 is τ_1 and with L and N_1 is τ_2, then

$$a=\tau_1\,,\qquad b=\tau_1-\tau_2$$

If we need only two significant figures for the value of L, we may construct another nomogram following:

The equation (2) is rewritten as

$$\frac{1}{N_0N_2}-\left(\frac{1}{N_0}+\frac{1}{N_2}\right)\frac{1}{L}+\frac{2}{N_1L}-\frac{1}{N_1^2}=0$$

or

$$\begin{vmatrix} d & d\left(\lambda+\frac{\mu}{N_0}\right) & 1+\nu\left(\lambda+\frac{\mu}{N_0}\right)^2 \\ d & d\left(\lambda+\frac{\mu}{N_2}\right) & 1+\nu\left(\lambda+\frac{1}{N_2}\right)^2 \\ d & d\left(\lambda+\frac{\mu}{L}\right) & 1+\nu\left(\lambda^2+\frac{2\lambda\mu}{L}+\mu^2\left(\frac{1}{N_1L}-\frac{1}{N_1^2}\right)\right) \end{vmatrix}=0,$$

where λ,μ,ν and d are arbitrary constants.

Hence the equation of the axis of variable N_0 and N_2 is

$$x=\frac{d}{1+\nu\left(\lambda+\frac{\mu}{\theta}\right)^2},\quad y=\frac{d\left(\lambda+\frac{\mu}{\theta}\right)}{1+\nu\left(\lambda+\frac{\mu}{\theta}\right)^2},\qquad \theta=N_0,\ N_2$$

for variable L: $\quad y=\left(\lambda+\frac{\mu}{L}\right)x$

for variable N_1: $\quad \left\{1-\nu\left(\lambda+\frac{\mu}{N_1}\right)^2\right\}x+2\nu\left(\lambda+\frac{\mu}{N_1}\right)y=d$

(The family of these N_1 lines envelopes the axis of N_0 and N_2).

CHART III has been thus drawn.

§ 3 Application and accuracy

We take the growth of population of U. S. A. for example. According to the Bureau of the Census:

Year	Popul. (million)	Year	Popul. (million)	Year	Popul. (million)
1800	5.31	1860	31.44	1920	105.71
10	7.24	70	39.82	30	122.78
20	9.64	80	50.16	40	131.67
30	12.87	90	62.95	50	150.70
40	17.07	1900	75.99		
50	23.19	10	91.97		

Taking 1850 year as initial, and 10, 20, 30, 40, 50 years for time intervals, we obtain the value of L using chart I, and the value of a and b using chart II following:

N_1	N_1	N_2	$n=N_1^2/N_0$	$n-N_2$	N_2-N_1	L_0	L_0*
23.2	31.4	39.8	42.5	2.7	8.4	26.2	26.15
23.2	39.8	62.9	68.5	5.6	23.1	95.0	99.24
23.2	50.2	92.0	109	17	41.8	105	105.12
23.2	62.9	122.8	171	48	58.9	72.6	72.68
23.2	76.0	150.7	250	99	74.7	56.5	56.79

L	L*	$\tau_1=a$	τ_2	$b=\tau_1-\tau_2$	(b) per year	τ_1*$=a$*	τ_2*	b*
66	65.96	0.610	0.089	0.521	0.0521	0.612	0.095	0.517
157.9	162.14	1.770	1.088	0.682	0.0341	1.790	1.123	0.667
197	197.12	2.025	1.080	0.945	0.0315	2.014	1.074	0.940
195.4	195.48	2.020	0.736	1.284	0.0321	2.005	0.746	1.259
207.2	207.49	2.110	0.539	1.571	0.0314	2.078	0.549	1.529

* Asterisk stands for the value by calculation.

From these data we see that the error of these charts is about 2%.

Supplementary note

If ratios of the number of individuals are introduced as arguments we can construct nomograms to find the value of a or b separately using following equations, which are reduced from equation (1).

$$e^{a}=\left(\frac{1-u}{u}\right)^{2}\cdot\left(\frac{w}{1-w}\right)$$

$$e^{-b}=\left(\frac{u}{1-u}\right)\cdot\left(\frac{v-1}{v}\right)$$

where $u=\frac{N_0}{N_1}$, $v=\frac{N_2}{N_1}$, $w=uv=\frac{N_0N_2}{N_1^2}$

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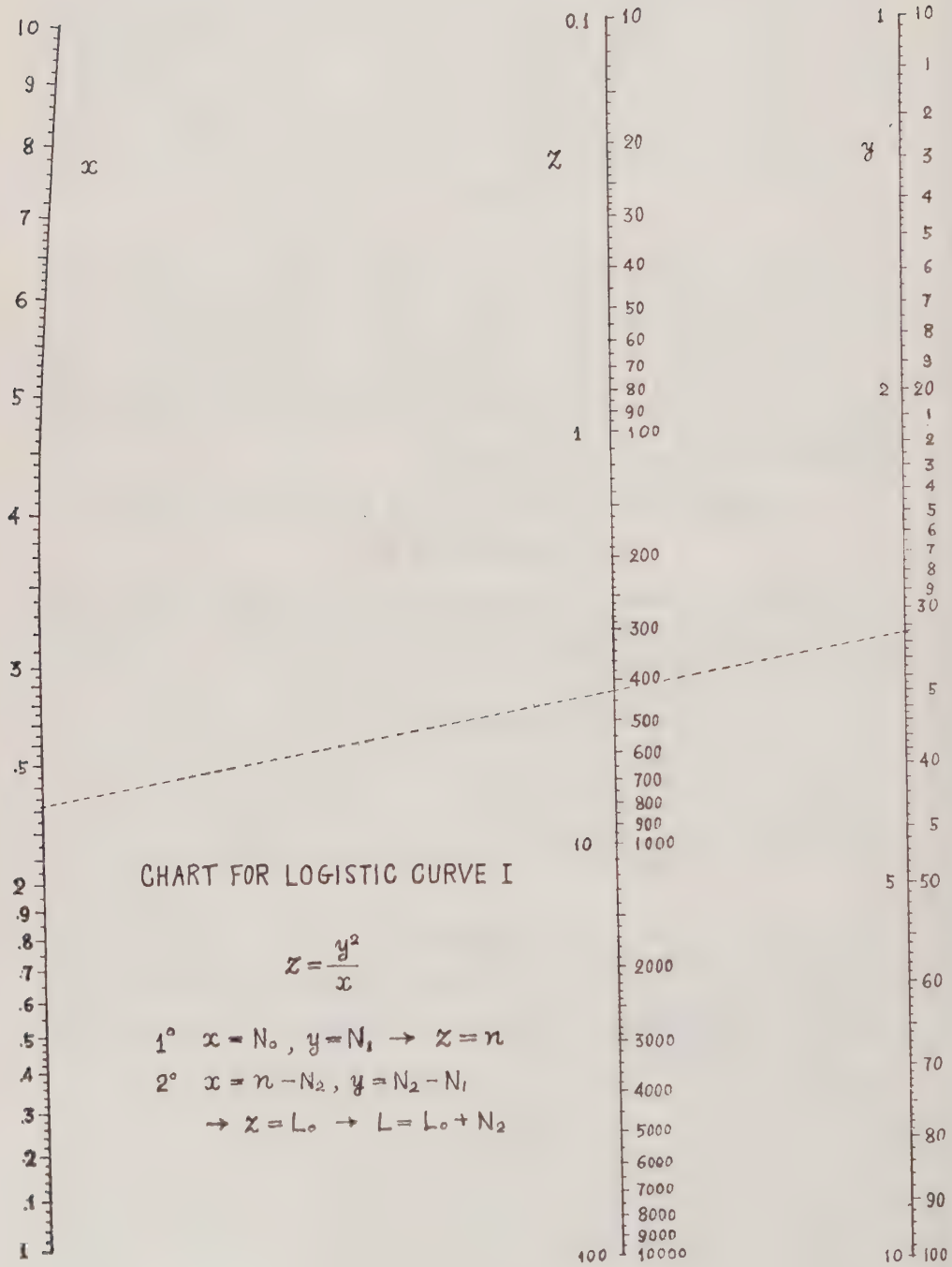
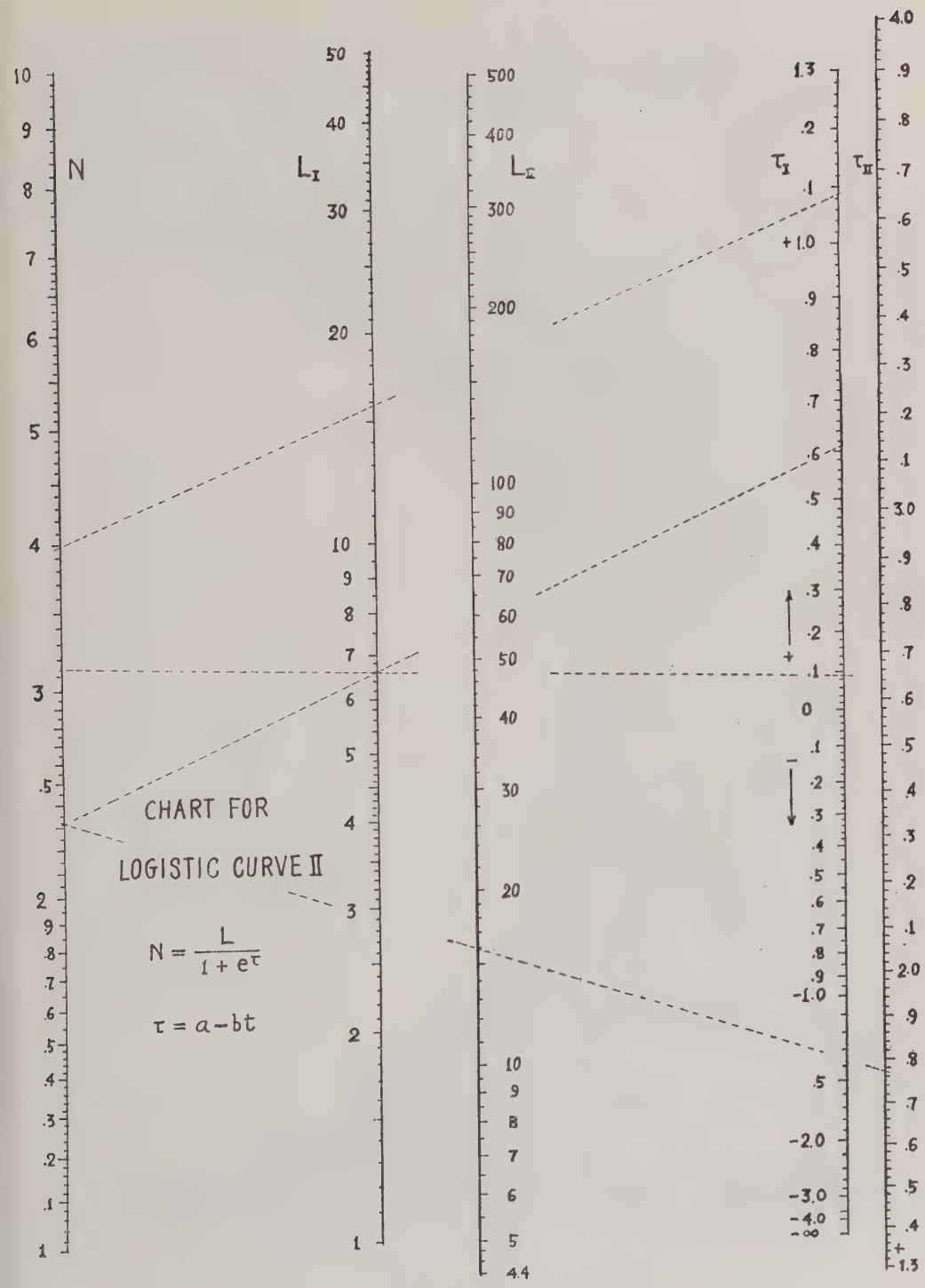

x
z
y
CHART FOR LOGISTIC CURVE I
$z = \frac{y^2}{x}$
1° $x = N_0$, $y = N_1$ → $z = n$
2° $x = n - N_2$, $y = N_2 - N_1$
→ $z = L_0$ → $L = L_0 + N_2$


N
L_I
L_{II}
τ_I
τ_{II}
CHART FOR
LOGISTIC CURVE II
$N = \frac{L}{1 + e^{\tau}}$
$\tau = a - bt$

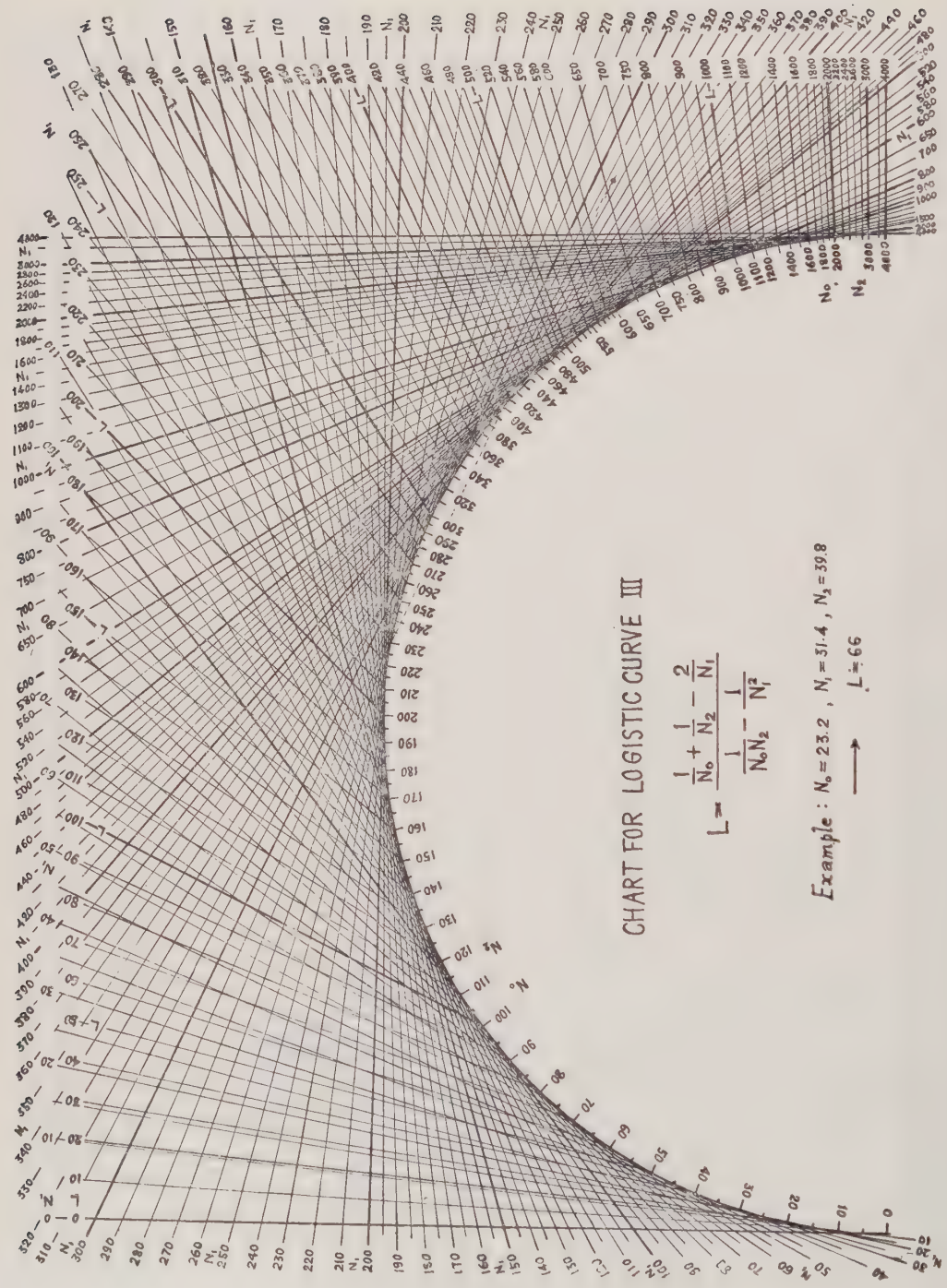


CHART FOR LOGISTIC CURVE III
$L = \dfrac{\frac{1}{N_0} + \frac{1}{N_2} - \frac{2}{N_1}}{\frac{1}{N_0 N_2} - \frac{1}{N_1^2}}$
Example : $N_0 = 23.2$, $N_1 = 31.4$, $N_2 = 39.8$
$\longrightarrow$ $L = 66$

Journal of the Tokyo University of Fisheries, Vol. 46, Nos. 1–2, 1960

STUDIES ON THE FAT METABOLISM OF FISH MUSCLES
4. EFFECTS OF THE COMPONENTS IN FOODS ON THE CULTURE OF RAINBOW TROUT

Toyoki Ono, Fumio Nagayama and Tsumoru Masuda

(With Plates 16–18)

(Received Feb. 15, 1960)

We find often a certain disease of rainbow trout which occurred in the all stages through the fish culture. The diseased fish are generally more dark color than normal, and when come up to the advanced stages of the disease float at the surface of the water. The gills of diseased fish, however, are much lighter than normal and in extreme cases these may show a very pale pink color. This discoloration is due to a marked anemia, which is one of the most character istic symptoms of the disease.

The external appearance of liver is the most striking feature of the diseased fish, which typically have fatty livers with light yellow or yellowish gray color. This may be due to the deposition of lipoids in the liver cells, in where lipoid replaced glycogen. Such deposition of lipoids in severe cases give a lipoid degeneration of liver cells, and consequently the fish become so much lean as ended in death.

The disease is considered to be caused by a mulnutrition which results from overfeeding or the use of unsuitable diets. However, some investigators have opinions that it may be caused by certain becteria or virus. German invstigators have found that the disease is more likely caused by the use of certain foods, especially these rich in fat. Recently the increase of the disease at hatcheries in the United States has been considered to be caused by the more extensive use of vitamin-dificient dry meals with too little fresh meat in the diet.

In 1929 Gaschatt found that low temperatures of water are an important factor which brings on the outbreak of the disease, and in the United States of America it appears to be most prevalent during cold weather. In Japan, however, these opinions are not always reasonable at the point of view introduced from the fact that the death rate of the diseased fish was more rather higher at 12～13°C. of the water temperature than the lower temperatures as 8～11°C. in the period of December～February.[1] In fact, most of culturists in Japan believe that the disease with the lipoid degeneration of liver may be caused by feeding of the unsuitable diets made from the materials stored for long time as dry shrimps, chrysalis meal, SP meal etc., which may contain some unknown components possible to retard the growth of fish.

We intend to investigate the relations between the disease and the components of rainbow trout diets. In this paper we present the results obtained from the experiments concerning with the effects of the fat contents and thier qualities in foods on an outbreak of disease.

EXPERIMENTAL

1. Fingerlings of rainbow trout

In this experiment were used the fingerlings which were spawned in spring of 1959 at the Okutama Hatchery and reached an average weight 1.35 grams. In one hatching trough were kept 400 fingerlings, and 8 troughs were prepared at same time for rearing.

2. Artificial foods

The fish foods was prepared by the following procedures. First, the various foods which are used by most of culturists in Japan were perfectly freed from the fatty substances through the benzole extraction of materials, owing to observe the subjects concerning with the components of fat. Next, was prepared a desirable diet contains fats and oils for the experiments, which based on the food free from fatty substances. Diets for experiments had the following percentage composition.

1) Control section (Diet number 1). Dry chrysalis powder, (CP) 20: flour F), 20; SP meal, 10; frozen goby (FG), 30; frozen mysid shrimp, (FMS) 20.

2) Second section (Refined salmon oil sec., diet number 2). CP (free from fat), 16.7; F, 16.7; SP (free from fat), 16.7; skim milk (SM), 4.2; fresh polk liver (FPL), 16.7; refined salmon oil (RSO), 8.1; dry mysid shrimp (DMS) free from fat, 16.7; mineral and vitamin mixtures (MV), 4.2.

3) Third section (Section containing an excess of salmon oil, diet number 3). CP (free from fat), 14.8; F, 14.8 ; SP (free from fat), 14.8; SM, 3.7; FPL, 16.7; RSO DMS. (free from fat), 14.7 ; MV, 3.7.

4) Fourth section [Section containing the oxidized salmon oil (OSO), diet number 4]. The diet of this section contained 8.1% of OSO in place of the refined salmon oil in diet No. 2 except the other compositive foods.

5) Fifth section (Section containing an excess of OSO, diet number 5). This section contained 16.7% of OSO in place of RSO in diet No. 3.

6) Sixth section (Sperm head oil section, diet number 6). Contained 8.1% of sperm head oil in place of the RSO in diet No. 2.

7) Seventh section (Section containing the rancid dry shrimp, diet number 7). CP 10, F 20, SP meal 10, FG 20, FMS 10, rancid dry shrimp 30.

8) Eigth section (Section containing the frozen pacific jack mackerel with much appearances of rancidily, diet number 8). CP 10, F 20, SP meal 10, FMS 20, frozen pacific jack mackerel 30.

After the foods were mixed with each section's composition as above mentioned, they should be supplied to fish as soon as possible (section number of the diet corresponds to experimental group number). If fish products are uses, they should not be mixed with other foods until the time of feeding, because of the quick spoilage of fish products. The food requirements of fish were determined by experiences to take a quantity corresponding 10% of fish weights, although there is somewhat a difference on the exact ration for requirement of diets at each fingerling stage. As shown in Table 1, the chemical composition of food represented many differences on the components, especially on protein, fat and volatile basic com-

Table 1. Chemical composition of rainbow trout foods

Rainbow trout foods	Moisture	Fat	Protein	Carbohydrate	Ammonia -N	Amine -N
	(%)	(%)	(%)	(%)	(mg. %)	(mg. %)
Chrysalis meal	9.3	17.5	59.8	–	24.7	0.97
SP meal	14.3	8.6	34.5	9.6	577	39.7
Dry shrimp	27.9	2.3	60.9	2.2	160	0.96
Frozen mysid shrimp	84.6	0.54	11.1	1.5	96 3	6.09
Frozen goby	78.2	1.1	18.7	0.3	–	–
Frozen pacific jack mackerel	75.0	3.0	20.0	0.7	–	–
Skim milk	4.2	1.0	34.8	52.2	–	–
Flour	14.4	0.9	7.5	75.7	–	–
Pork liver fresh	72.8	4.4	19.5	1.8	–	–

Table 2. Characteristics of the oils added in diets and the ether extracts from foods.

Oils and ether-extracts	Iodine value	Acid value	Peroxide value	
			Before exp.	After exp.
Refined salmon oil$^{\triangle}$	138.8	0.64	6.4	–
Oxidized salmon oil*$^{\triangle}$	94.6	2.13	110.0	111.0
Sperm head oil$^{\triangle}$	66.2	1.59	4.8	–
Chrysalis extracts	140.9	39.1	3.7	18.9
Dry shrimp ext.	56.3	90.0	16.8	37.3
Mysid shrimp ext.	217.6	217.0	4.6	41.5
SP meal ext.	40.3	53.7	9.3	16.8

$^{\triangle}$ ••• Stored in amples, * ••• Oxidation by air blowing at 100°C. for 40 hours.

pounds. The latters, amine-and ammonium nitrogen, were contained much amounts in SP meal than all other foods. However, in those diets prepared by the mixture of various foods no differences were found in the ammonia contents (Table 3).

Characteristics of oils and ether extracts were shown in Table 2. From the results it was evident that the oxidized salmon oil had a very high peroxide value in spite of the lower acild value. On the contrary, the ether extracts ob tained from such foods as chrysalis meal, dry shrimp and SP meal had higher acid value, but the peroxide value was not so much as the case of oxidized salmon oil. However, the peroxide matters have such specifity as derived from oil during the proceeding of oxidation of fatty acids.

Therefore, it was obvious that the artificial diets prepared by the addition of refined salmon oil, oxidized salmon oil and sperm head oil increase always in the acid value and peroxide value, especially in the latter, compared with the original oils. These facts may be caused by the oxidation of fat which is carried out by the physical treating of foods for mixture and by storage of diets.

Table 3. Chemical composition of diets used in raring.

Diet number	Moisture	Fat	Protein	Carbo-hydrate	Ash	NH -N	Amine -N
	(%)	(%)	(%)	(%)	(%)	(mg%)	(mg%)
1	56.5	3.16	18.9	19.8	1.65	55.8	5.71
2	47.3	8.18	23.0	15.9	5.26	–	–
3	44.1	12.12	22.6	15.7	5.44	–	6.12
4	46.3	2.82	23.0	17.3	5.59	47.2	6.57
5	44.9	13.22	21.8	16.2	4.88	46.2	6.76
6	46.9	7.16	23.6	16.8	5.53	49.0	5.63
7	59.4	2.12	17.8	14.9	5.80	53.8	3.09
8	53.9	4.28	19.8	19.0	2.50	63.8	3.31

Table 4. Characteristics of the ether extracts from diets.

Diet number	Iodine value	Acid value	Peroxide value
1	134.5	96.5	268
2	135.3	30.8	153
3	136.3	18.6	286
4	124.1	31.6	836
5	125.4	18.6	1360
6	82.7	22.5	137
7	116.5	89.6	1030
8	129.7	77.7	462

RESULTS

1. Growth and disease of rainbow trout

One section as an experimental unit in a trough involved 400 fingerlings of average weight of 1.35 grams. Experiments for rearing were carried out from June 9 to August 22, and calculated the ratio of death to total fish counting every week on the number of servived fish in trough. The ratio of death or degree of growth was shown in Table 5. From the results it was evident that the number of diseased fish represented many differences among the effect of diets, namely the total number of dead fish reached to as much as 62.5%, 55.5% and 47.8% respectively in the groups V, VII and VIII. On the contrary, the group VI showed so lower as only 5.8%, and the group I (the control group) gave a lower value as 16.3% (Table 5).

This may be attributed to the feeding of such rancid foods as dry shrimp meal or artificial diets contained the oxidized salmon oil, and an undesible component in diet which is seemed a sort of peroxide compound will bring about the disease. On the other hand, it was found that the death number of fish in the groups II and III was greater than that of group I in spite of containing the non-oxidized

Table 5. Effect of diets on the growth.

Exper. group		1	2	3	4	5	6	7	8
Number of fingerlings at starting of exper.		400	400	400	400	400	400	400	400
Average weight of fish at starting of exper. (g)		1.35	1.36	1.35	1.33	1.35	1.37	1.34	1.39
Number of daed fish	1st week (June 9–15)	0	5	3	4	4	6	3	0
	2nd week (June 16–22)	1	8	4	3	1	6	1	0
	3rd week (June 23–29)	4	10	9	5	13	4	12	0
	4th week (June 30–Aug. 5)	3	7	13	16	57	3	33	3
	5th week (Aug. 5-12)	8	16	33	44	117	2	78	29
	6th week (Aug. 13–19)	27	30	32	65	43	2	85	121
	7th week (Aug. 20–22)	22	15	9	27	15	0	10	38
Nos. of servived fish		335	309	297	236	150	377	178	209
Nos. of dead fish		65	91	103	164	250	23	222	191
Rate of death (%)		16.3	22.8	25.8	41.0	62.5	5.8	55.5	47.8
Average weight of fish at the closing of exper. (g)		4.64	4.48	5.08	5.03	6.54	3.79	6.38	5.82

oil in the formers. This may causes the deficiency of an available component in diet. However, it was interested that the group VI in which was used sperm head oil showed the lowest reduction of fish among all eight experimental groups. This may be due to presence of an available component preventing a disease as the lipoid degeneration from rainbow trout fingerlings.

2. Effct of ammonia and amide

Brockway (1950) pointed out the importance of effects of ammonia on fish in the demonstration of metabolic products and their effects, and explained that a concentration of 1 p.p.m. of NH_3 in the water makes the fish actually to suffer from suffocation owing to the inability of the hemoglobin in blood to absorb oxygen or to liberate CO_2.

In the present experiments, however, the influence of NH_3 and amines in diets made no appearances on suffocation in rearing of fingering of rainbow trout, in reaso nof much smaller amounts of ammonia or amine solved in the water. Why occours the lipoid degeneration by the addition of dry shrimp? Details of this problem will be discussed in following section.

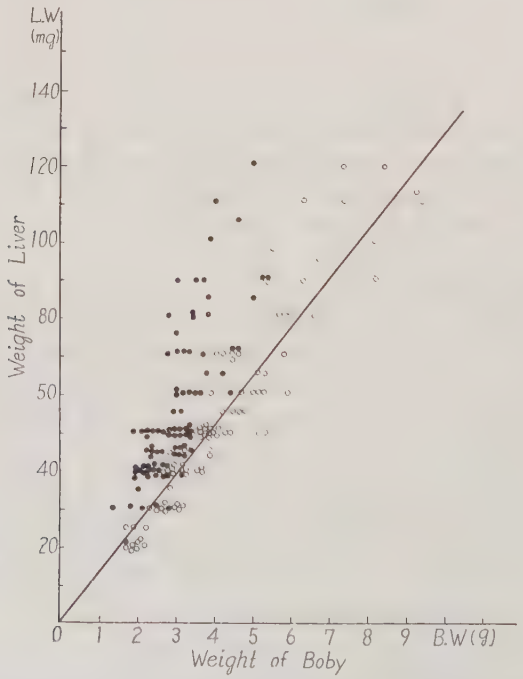
3. Appearances of dead fish

The samples (dead fish) taken from groups IV V, VII and VIII all not contain-

Table 6. Appearance of dead fish.

Exper. group	Number of fish	Appearance of death fish
I	4	No food in stomach up to 100%; lean fish; no color change on liver
II	5	No food in stomach up to 80%; yellowish liver
III	8	No food in stomach up to 50%; yellowish liver up to 63%
IV	16	No food in stomach up to 59%; gray gill; yellowish liver up to 94%
V	40	No food in stomach up to 70%; gray gill up to 88%; yellowish liver up to 100.
VI	2	Lean fish; no color change on liver
VII	29	No food in stomach up to 55%; gray gill up to 65%; yellowish liver up to 83%
VIII	3	No food in stomach up to 100; gray gill; yellowish liver up to 100%

ed any food in their stomachs and the gills became even grayish white so to be recognized as anemia (See Plate No. XVI, Figs. 1 and 2). As the most striking feature of this disease is the appearance of the liver, this experiment was based also typically on the discoloration of liver. On the contrary, in the case of groups I and VI they had no any symptom of disease.



Text-Fig. 1. Relation between liver weight and body weight.
● diseased fish ○ healthy fish

4. Anemia

(a) Number of the red blood corpuscles.

At the closing of rearing, 10 fingerlings are picked up from each trough and the number of the red blood corpuscles was counted. As shown in Table 7, the number of anemic fish increases in order of the groups VIII, VII and V and were so much reduced in the number of red blood corpuscles as 456,000, 481,000 and 537,000 respectively per cubic mm of blood. However, the fish of the group VI indicated the nomal red blood composition with average number as 1,268,000.

(b) Color of gills.

A judgment of anemia was organolepticall carried out by the observation of a color of gills. This method gave the results as same as the counting method of red blood corpuscles (see Table 8). Compared Table 7 with Table 8, it was proved that

Table 7. Number of the red b'ood corpuscles.

Exper. group	I	II	III	IV	V	VI	VII	VIII
Aver. No. of R.B.C	801,000	657,000	725,000	553,000	537,000	1,218,000	481,000	456,000
Above 1,000,000/mm² of RBC (%)	40	20	10	10	10	90	0	0
Below 500,000/mm² of RBC (%)	10	30	10	40	60	0	70	90

Table 8. Red bood corpuscles judged by the color of gills

Exper. group	I	II	III	IV	V	VI	VII	VIII
Number of sample	317	254	186	172	52	264	53	104
Above 1,000,000/mm² of RBC (%)	38	29	15	5	8	93	29	10
Below 500,000/mm² of RBC (%)	20	33	53	73	73	3	51	67

certain number of healthy fish being not anemic is present even in the groups V, VII and VIII. Such an inconsistency on the experiments may be sometimes caused by the complexity of fish culture.

5. Weight of livers

We observed that the livers of diseased fishes were always more fatty than the other fish. From this observation it will be able to produce an evidence in support of the conclusion of disease with the lipoid degeneration (Text-Fig. 1).

6. Chemical composition of liver and muscle.

Picking up 50 living fishes from each rearing trough at August 13, deter-

Table 9. Comparison of chemical composition of liver and muscle of rainbow trout.

Tissue	Exp. group	Moisture	Fat		Glycogen		Phosphorus		Iron		Phosphorus*	
			Fresh matter	Dry matter	Fresh m.	Dry m.	Fresh m.	Dry m.	Fresh m.	Dry m.	Eher extract	Dry m.
			(%)	(%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)
Liver	I	76.9	2.1	9.1	219	940	212	917	4.74	22.6	250	22.7
	II	80.8	2.4	15.0	–	–	212	1100	5.86	36.0	–	–
	III	80.8	2.9	12.3	–	–	223	1160	11.06	65.8	71.4	10.8
	IV	81.8	2.7	14.9	–	–	194	1070	5.46	35.3	178	26.4
	V	84.2	3.1	14.5	30	158	173	1090	10.02	74.2	96	18.8
	VI	79.7	1.6	7.7	107	527	206	1010	9.13	37.8	63.6	5.01
	VII	79.3	2.6	13.0	48	233	212	1020	2.65	14.8	172	21.6
	VIII	76.2	4.3	20.5	28	88	221	928	–	–	118	21.3
Muscle	I	78.8	2.2	10.3	10.9	51.4	197	931	3.55	18.7	610	63.3
	II	80.2	2.7	13.8	–	–	166	837	1.69	9.90	577	78.6
	III	79.3	3.2	15.3	8.8	42.5	171	824	1.37	7.87	–	–
	IV	79.9	3.3	16.3	–	–	160	797	1.44	8.61	476	78.1
	V	79.4	3.4	16.3	0	0	183	887	2.01	11.7	600	99.0
	VI	81.1	2.4	12.5	–	–	180	950	1.46	8.82	300	38.0
	VII	79.6	2.5	12.1	8.5	41.6	192	940	2.07	11.6	2260	277
	VIII	80.3	2.5	12.7	8.5	22.8	180	916	3.69	21.5	1170	148

* Indicate the fat soluble type.

Table 10. Distribution of TCA soluble type phosphorus (mg. % in fresh matter).

Tissue	Exp. group	Inorganic phosphorus	Heated for 10 minutes with *N*-HCl	$\triangle_{10}P$	Heated for 180 minutes with *N*-HCl	$\triangle_{80}P$	$\triangle_{80}P-\triangle_{10}P$
Liver	I	39.3	40.3	1.0	49.4	10.1	9.1
	II	–	–	–	–	–	–
	III	34.8	41.2	6.4	46.6	11.8	5.4
	IV	–	–	–	–	–	–
	V	50.7	52.8	2.1	60.9	10.2	8.1
	VI	41.0	41.3	0.3	52.2	11.2	10.9
	VII	5.6	5.6	0	8.4	2.8	2.8
	VII	33.5	36.5	3.0	44.2	10.7	7.7
Muscle	I	28.5	40.2	11.7	46.8	18.3	5.4
	II	–	–	–	–	–	–
	III	30.0	39.4	9.4	44.0	14.0	4.6
	IV	–	–	–	–	–	–
	V	33.8	37.8	4.0	44.2	10.4	6.4
	VI	30.6	42.2	11.6	44.2	13.6	2.0
	VII	34.2	40.8	6.6	40.2	7.6	1.0
	VIII	29.8	34.4	4.8	35.8	6.0	1.2

mined the following components in livers and muscles; moisture, fat, glycogen, phosphorus, iron and phosphatides. As shown in Tables 9 and 10, there was a considerable difference on the content of each component, especially on that of fat and glycogen. Such features in muscles, however, were not able to detect except phosphatides which were contained in much more amounts in diseased fishes than healthy ones.

7. Histological observations on the diseased and healthy rainbow trout.

1) *Distributoin of glycogen in liver.*

Stained liver slices by Bauer-Feulgen's method[3] and we can discriminate glycogen from other matters using a microscope throughout the stained tissue which become red purple with glycogen and purple with the latters. This feature agreed with the data obtained from the chemical analysis (Plate No. XVII).

2) *Distribution of fat in liver*

Stained a slice prepared from frozen material by Jackson's method[4] using the Carbol-Sudan III solution. Fat was distinguished by the reddish orange color from other tissues which were stained light purple. In the case of the normal grown fish (healthy fish) as in the group I or VI, the fatty cell scattered among the common cell. On the contrary, the fatty cell in the group V or VII occupied a large area of tissue.

3) *Distribution of cholesterol in liver*

By Schultz's method[5] cholesterol was stained green. Through the observations by microscope, it will be demonstrated that more cholesterol distribute in the fish livers of the groups V and VII than the groups I and VI (Color plate No. XVI, Fig. 4).

4) *General histologial observations of tissue*

Liver, kidney and spleen were histologically observed after the staining by Ehrich's hematoxylin-eosin. (Plate No. XVIII)

(a) Liver tissue of fish in groups I and VI had a fine structure, but that in the groups V and VII had a coarse structure, because of the removal of the fat on preparation of a slice.

(b) The observations on kidney and spleen tissue gave little a satisfactory result.

Discussion

Most of fish-culturists in Japan were concerned for the diseases of rainbow trout. The diseases prevails to a large extent among pondfish and make a great reduction of fish, which has such an extreme case indicating up to 80% of the death rots of fish. Therefore, the diseases of fish constitutes one of the most important problems confronting the fish-cultrists and they desire to know by what reason occur such great majority of the infectious diseases of rainbow trout. We support the opinions that this disease is one of the malnutrition caused by the use of unsuitable diet, which have been considered by most of culturisis as a disease incident to rainbow trout.

Lipoid degeneration of the liver is one of the most striking features appeared on the diseased fish of which livers have less enzyme action concerned with fat metabolism. In this paper we pointed out that a sort of diet prepared

artificially made the outbreak of the lipoid degeneration of liver. The lipoid degeneration of liver may be caused by taking much amounts of an injurious component which will lead after all to the death of fish owing to the abnormal fat metabolism. We conclude from the present experiment that such an injurious component may be a sort of peroxide matter produced during the storage of diets. It is generally known that the peroxide matters in foods are the oxidized products of unsaturated fatty acids in ordinary fish oils. Therefore, the number of survived fish among the fingerlings fed with the diets prepared by the addition of oxidized oils, should be reduced In fact, we proved the possibility for prevention of disease in the experiments of rearing rainbow trout, but the experimental procedures for protection of fish from the disease has been not satisfactorily established.

Here, we can only report that there was a certain fish oil to prevent the lipoid degeneration of liver with a normal growth of fish, it was sperm head oil. Experiments to discriminate an effective component in sperm head oil is being contiuued.

SUMMARY

Experiments were carried on the rearing of rainbow trout using diets which were combined with the various composition of food. Even at a fingerling stage, there occurred the disease showing such characteristic symptom as lipoid degeneration of liver, anemia, abnormal tissue structure in kidney, and deposition of cholesterol.

The disease might be caused by taking of the peroxide matters which occurred in food through the oxidation of fatty substances. This conclusion was established by the experiments that the diets containing the artificially oxidized salmon oil made fish lipoid degeneration of liver as same as common unsuitable diets.

A special diet containing sperm head oil was able enough to prevent the outbreak of disease, but the origin has not been clarified.

Histological observations of the diseased fish were made on the distributions of glycogen, fat and cholesterol in the liver. There was seen always a marked increase in fat and decrease in glycogen. Cholesterol was accumulated in dieased fish liver.

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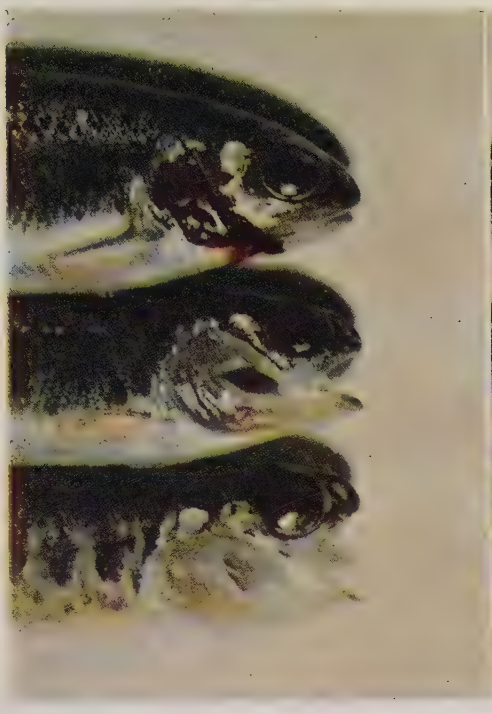

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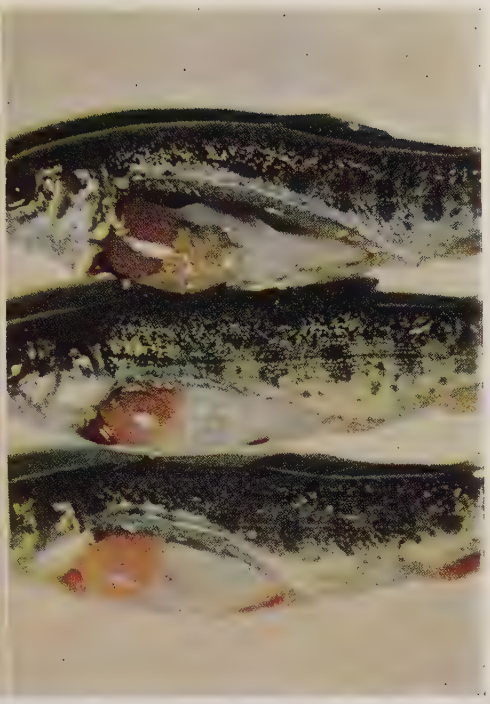

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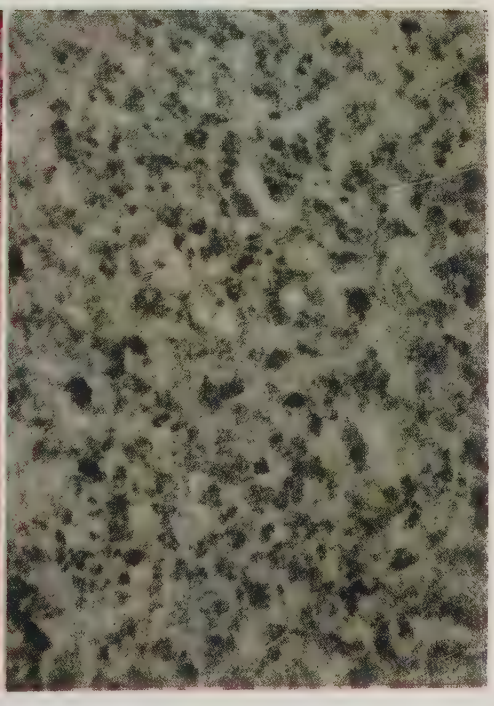

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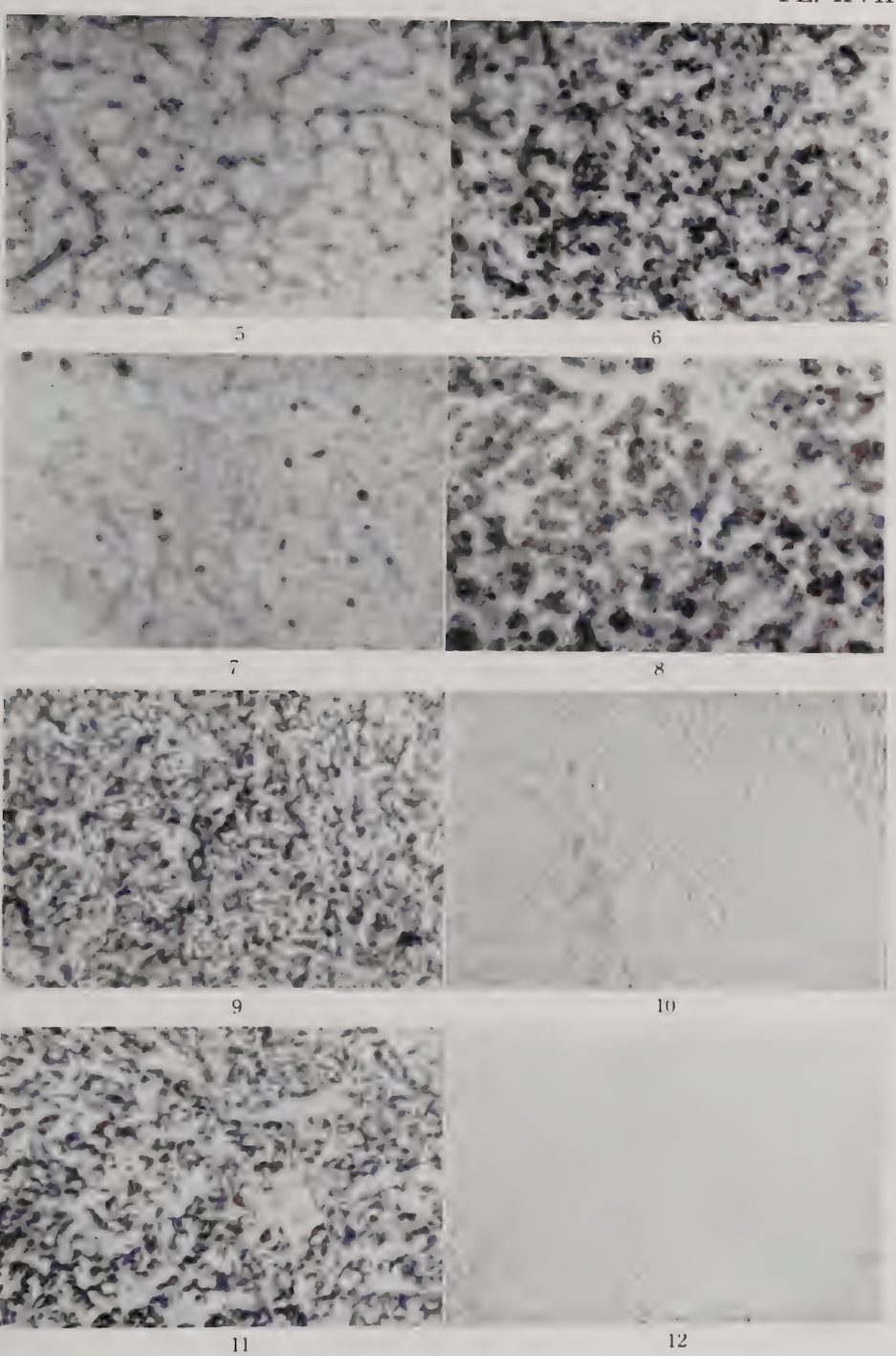
PLATE XVI

Fig. 1. Color of gills. Group I shows normal color of gills (upper); groups V and VII show severe anemia (median and lower).

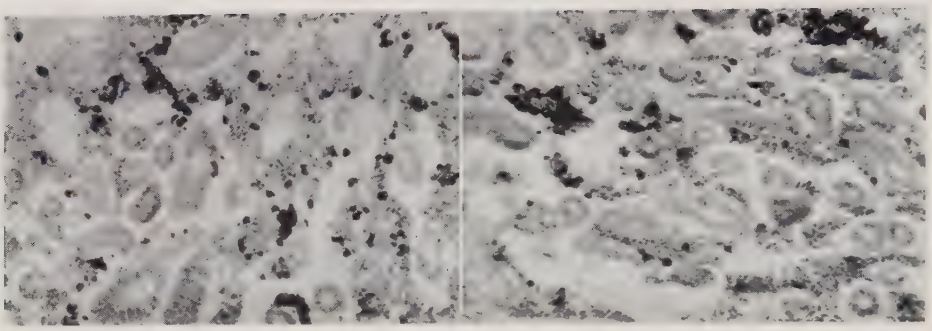
Fig. 2. Color of liver. Group I shows normal color of liver (upper); groups V and VII show yellow discoloration of liver (necian and lower) Striking congestions or hemorrhage around anus and at anal fin are observed on group V and VII.

Fig. 3. Lipid in liver of group VII (Acetic carbol sudan III, Ehrich's acid hematoxulin, double stain).

Fig. 4. Cholesterol in liver of group VII. Also group V is positive to the reaction, but negative with groups I and VI. (Schultz's reaction).



Figs. 5 to 8. Lipid in liver (Acetic Carbol-Sudan III, single stain).
Fig. 5. Group I.
Fig. 6. Group V.
Fig. 7. Group VI.
Fig. 8. Group VII.
Figs. 9 to 12. Glycogen in liver (Bauer-Feulgen's reagent, single stain).
Fig. 9 Group I.
Fig. 10. Group V.
Fig. 11. Group VI.
Fig. 12. Group VII.



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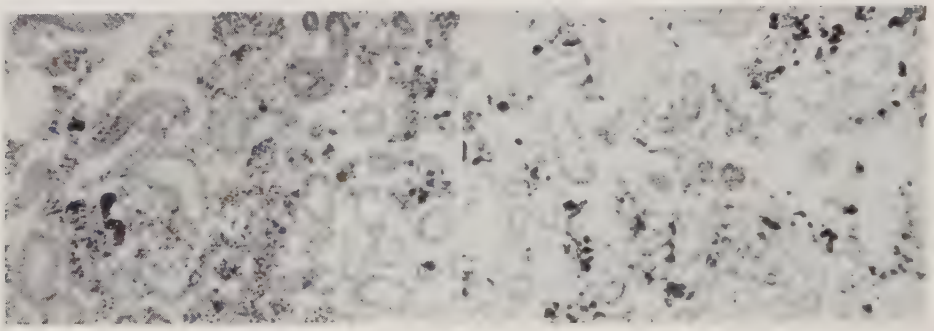

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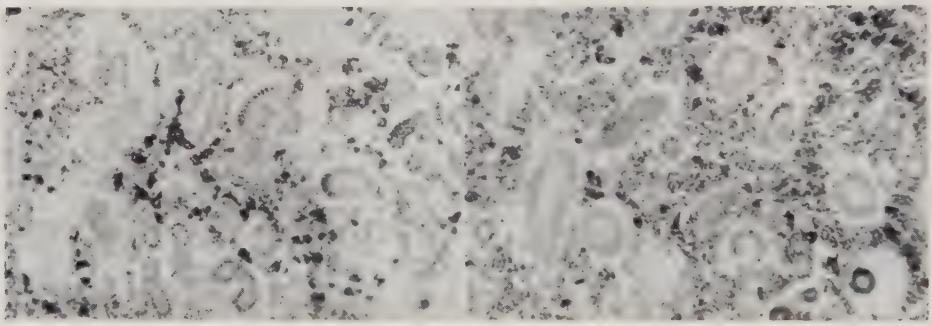

17 18

19 20

Figs. 13 to 18. Kidney (Ehrich's Hematoxylin-Eosin, double stain).
Figs. 13 and 14. Group I.
Figs. 15 and 16. Group V.
Figs. 17 and 18. Group VII.
Figs. 19 and 20. Liver (Ehrich's Hematoxylin-Eosin, double stain).
Fig. 19. Group I.
Fig. 20. Group VII. Gap among tissue is supposed to be a trace of lipid which was extracted with solvent.

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APPLICABILITY OF ANTIOXIDANTS TO MARINE PRODUCTS[1)–5)]

Kenzo Toyama, Kuman Saruya, and Kazuo Ando

With Plate 19

(Received Feb. 15, 1960)

As a whole, the oil of marine products contains large quantities of polyunsaturated fatty acids, and so it is well known that they are apt to autoxidize in the presence of air. Indeed, in many of the marine products, especially those stored at common temperatures and in the presence of air such as dried-fish or salted fish, it has been a serious problem to prevent their deterioration due to the oil oxidation, namely, rancidity and rusting. Since smoked fish rarely suffers from rancidity and rusting, early workers had expected that some components of smoke might have been effective in protecting these products[6)7)8)9)]. Thus pix liquida or pyroligneous liquid was applied to the marine products, with its faint efficacy recognized. But it has not been put to practical use. In recent times, on the other hand, as a result of research on the oil autoxidation mechanism, many excellent antioxidants were discovered. Their application was successful in preventing deterioration not only of oil and fats but also various kinds of oily products[10)11)] such as shortening oil, margarine, and soaps[12)] as well as fatty foods[13)14)], e.g., bacon, potato chips[15)], and crackers[10)16)].

The appearance of antioxidants brought also the time of improving the preservability of some marine products. As the authors made an experimental research for procedures of reasonable application of some known antioxidants to dried fish and salted fish, a few highlights in the results they obtained, i.e., efficacy against the rancidity and rusting, evaluation in commecial application, secondary effects on the products, etc., are presented in the following.

Experimental

I. Antioxidants.

The names of antioxidants and their abbreviations used in this work are listed as follows:

BHA[10)11)17)] : Butyl hydroxyanisole, a mixture of 2- and 3-tert-butyl-4-hydroxyanisole isomers, corresponding to a product of Universal Oil Products Co., U.S.A., with a trade name of Sústane 1-F.

BHA": A mixture of butyl hydroxyanisole (67%), propyl gallate (20%), and citric acid (10%), corresponding to a product of Universal Oil Products Co., U.S.A., with a trade name of Sústane 3-F.

NDGA[18)]: Nordihydroguaiaretic acid, a product of Wm. J. Stange Co., U.S.A.

NDGA": A mixture of nordihydroguaiaretic acid (75%) with citric acid (25%) prepared in our laboratory.

EP[19)20)]: Ethyl protocatechuate, a product of Takasago Koryo Co., Japan.

PG[21)22)]: Propyl gallate, a product of Takasago Koryo Co., Japan.
IAG''[22)]: A mixture of iso-amylgallate (80%) and citric acid (20%), a product of Yoshitomi Seiyaku Co., Japan, with a trade name of Linton C.
BHT[24)25)26)]: Dibutyl-p-hydroxytoluene, a product of Universal Oil Products Co., U.S.A., with a trade name of BHT.
PL[6)7)]: Pix liquida, a product of Sankodo Co., Japan.

II. Method of application of antioxidants.

When applying antioxidants to marine products, it would be advantageous to utilize an existing equipment available in manufacturing marine products without preparing any special one.

In the processes generally adopted for making dried fish, the raw material is allowed to be cured for a specified time in NaCl solution or other curing agents. The authors added the antioxidants in this curing period. Since most of the antioxidants were either insoluble or only slightly soluble in aqueous solutions, it was necessary to prepare an emulsion by adding a concentrated alcoholic solution of antioxidant while vigorously stirring the curing solution.

In the case of dry salting, such as the preparation of *Aramaki* or *Kairyo-aramaki*, wherein no curing solution need to be used, a desired amount of antioxidants required was very minute in quantity, it was difficult to produce a uniform mixture. Consequently, the total required quantity of antioxidant was first mixed thoroughly with a small amount of common salt, then this mixture was diluted with increasing amount of the salt until the desired concentration of antioxidant was attained.

III. Preparation of samples.

(1) Salted-and-dried saury

Defrosted saurys were split down the back to remove the head and viscera, and then lightly washed to remove the blood. Pieces of saury flesh obtained from 13 fish individuals and weighing 940 grams in total were cured for 16 hours in a 7.5% NaCl solution (in earlier tests 12% NaCl was used) containing a known quantity of antioxidant. After curing, the fish were lightly washed again and dried in the daylight until the moisture content of the fish reached approximately 40%. In preparations for storage test, some of these cured products were wrapped with paper and allowed to stand at room temperature during the preliminary test. But, in later comparative tests, one sample was stored in its original form in a dark place while another sample was chopped into pieces and stored in a container.

(2) Salted salmon.

(a) *Kairyo-aramaki* (ordinary type of salted salmon).

Frozen salmon, *Oncorhyncus keta*, were used after removing the viscera and gill arches. In the preliminary tests, a fresh pink salmon, *Onkorhyncus*

golubusha was used. Samples from the same batch were salted using various antioxidants, respectively. The weight of curing agent (salt plus antioxidant) used amounted to 40% of the weight of the fish sample (30% in the preliminary test). The samples having been salted for 10 days (5 days in the preliminary test) were removed of adhering salt and then re-salted with common salt amounting to 10% of the initial weight of the fish sample. The products were stored in a temperature range of 4～13°C. (-5～0°C. in the preliminary test).

(b) *Aramaki* (mildly salted salmon).

Fresh salmon, *Oncorhyncus keta*, were used after removing the viscera and gill arches. Each batch contained 37.5 kg. of raw fish salted with salt and antioxidant. The amount of salting salt corresponded to 25% of fish weight. After salting for 1 day at room temperature, the products were stored in a chilling room at a temperature of -8～-15°C.

IV. Evaluation of efficacy of antioxidant.

(1) General

The quality of antioxidant applied to the fish, was organoleptically evaluated by observing the changes in color, odor, and taste of the products continuously in a period from the time of preparation of fish samples until the end of their storage.

The protective efficacy against the rancidity and rusting of the marine products was examined not only in an organoleptic manner as mentioned above but also by chemically analyzing the extracted oil as explained below.

(2) Oil extraction method.

(a) *Salted-and-dried saury.*

The sample was removed of the vertebra and cut into small pieces. Anhydrous sodium sulfate was added by an amount equal to 2 or 3 times the weight of the fish, mixed thoroughly, and then the oil was extracted by the SOXHLET method using peroxide-free ethyl ether. The solvent was removed as completely as possible under reduced pressure, and the oil was subjected to chemical test.

(b) *Salted salmon.*

The oil was extracted from the various sections of the product shown in

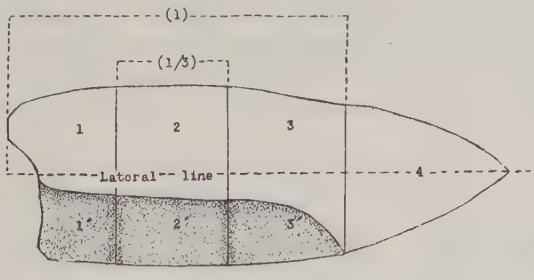

Fig. 1. Showing various parts of the sample body, from which oil was extracted.

Figure 1. The method of extraction was similar to that used for the salted-and-dried saury.

(3) Testing the oil properties.

Peroxide value was determined by a modified method of LEA[27] or "*Hot*" method of LEA.[28] The results are expressed as milliequivalent of peroxide oxygen per kilogram of oil. Oxidized acid was determined according to FAHRION's method.[29] The concentration of the oxidized acid in the oil is expressed as weight percent. Acid value determination was as follows: 1 gram of oil sample was dissolved in 20 ml. of an alcohol-ether mixture (1:4) and the solution was titrated with 0.1 N KOH alcoholic solution. The results are expressed as milligrams of KOH per gram of oil.

V. "Marginal use" of antioxidant.

In the case of oils and fats or their finished goods, the addition of a calculated necessary quantity of antioxidant can be performed with great ease. With marine products, however, the quantity of antioxidant necessary for positive effect has to go dissolved in the oils existing scattered throughout the body of these products. Consequently, it may be necessary to add larger quantities of antioxidant to the curing agents to ensure that the antioxidant is dissolved in the oils or oil tissue in such a sufficient amount as to prevent their deterioration. The optimum conditions must be experimentally determined for each specific marine product as to the amount of antioxidant dissolved in oil, the method of application, the antioxidant concentration in curing agent and the time of curing.

From the viewpoint of practice and convenience, it will be recommendable to determine the optimum amount of antioxidant on the weight proportion of antioxidant to the product or to curing agent, which can be readily known from the experiments that produced good storage, without regard to the oil content of the marine product.

When, however, too large concentrations of antioxidant are used, often the products acquire the specific odor or taste of the antioxidant or become discolored, the antioxidative effect being exerted in vain.

With this information, the authors selected the maximum concentration of the antioxidant within a range wherein the efficacy of the agent was not greatly weakened, and named this concentration "marginal use". When comparing the antioxidants with each other regarding their relative efficacy on marine products, they had respectively been applied in such a manner as to bring the antioxidant content in the products up to a maximum concentration selected as above.

Results

I. Salted-and-dried saury.

(1) Determination of marginal use of antioxidants.[1]

The "marginal use" of each antioxidant, BHA, BHA," NDGA, NDGA",

Table 1. The conditions of salted-and-dried saury treated with **BHA**.

No.	Concentration of BHA in NaCl curing bath (%)	(Days of storage of samples)*									
		(a=4, b=1)			(a=11, b=8)		(a=15, b=12)				
		Color	Odor of BHA	Taste of BHA	Acid value	Peroxide value	Color	Odor of BHA	Rancid odor	Taste of BHA	Rancid taste
1	0	yellow	−	−	17.0	296.4	reddish yellow	−	+	−	+
2	0.005	yellow	−	−	12.6	239.4	yellow	−	+	−	+
3	**0.010**	**tinged with light brown**	**∓**	**∓**	**14.0**	**66.6**	**yellow**	**−**	**−**	**−**	**∓**
4	0.025	tinged with light brown	+	+	11.6	51.4	yellowish brown	±	−	±	−
5	0.050	tinged with bluish brown	++	++	13.0	32.6	light bluish brown	+	−	+	+

* a=Days after the treatment; b=Days after the becoming dry of sample.
Average temperature 15±5°C.
(−): No symptoms observed. (+): Development of symptoms.

Table 2. The conditions of salted-and-dried saury treated with **BHA''**.

No.	Concentration of BHA'' in NaCl curing bath (%)	(Days of storage of samples)*										
		(a=4, b=1)			(a=12, b=9)							
		Color	Odor of BHA''	Taste of BHA''	Color	Odor of BHA''	Rancid odor	Taste of BHA''	Rancid taste	Acid value	Peroxide value	Oxidized acid
6	0	light yellow	−	−	yellowish brown	−	+±	−	±	13.1	103.8	4.21
7	0.005	tinged slightly with red	−	−	light yellowish brown	−	±	−	−	14.4	70.4	2.28
8	**0.010**	**tinged with light red**	**−**	**∓**	**light brown**	**−**	**−**	**−**	**−**	**12.8**	**41.2**	**2.04**
9	0.025	tinged with light red	±	±	light brown	±	−	±	−	13.7	33.0	1.72
10	0.050	tinged with red	±	+±	brown	±	−	+±	−	10.6	31.7	1.35

* a=Days after the treatment; b=Days after the becoming dry of sample.
Average temperature 9±5°C.
(−): No symptoms observed. (+): Development of symptoms.

Table 3. The conditions of salted-and-dried saury treated with **NDGA**.

No.	Concentration of NDGA in NaCl curing bath (%)	(Days of storage of samples)*								
		(a=6, b=1)			(a=12, b=7)			(a=22, b=17)		
		Color	Odor of NDGA	Taste of NDGA	Color	Rancid odor	Rancid taste	Acid value	Peroxide value	Oxidized acid
11	0	yellow	−	−	yellowish brown	+	+	12.0	89.7	14.02
12	0.005	yellow	−	−	tinged with red	±	+	10.0	73.6	15.60
13	0.010	tinged with light red	−	−	tinged with red	±	∓	12.2	83.2	6.60
14	**0.025**	**tinged with light red**	**−**	**−**	**tinged with red**	**−**	**∓**	**11.0**	**107.3**	**5.04**
15	0.050	tinged with red	−	±	tinged with dark	−	∓	11.9	101.9	5.56

* a=Days after the treatment; b=Days after the becoming dry of sample.
Average temperature 4±5°C.
(−): No symptoms observed. (+): Development of symptoms.

Table 4. The conditions of salted-and-dried saury treated with **NDGA''**

No.	Concentration of NDGA'' in NaCl curing bath (%)	(Days of storage of samples)*							
		(a=15, b=11)			(a=32, b=28)			(a=33, b=29)	
		Color	Odor of NDGA''	Rancid odor	Acid value	Peroxide value	Oxidized acid	Color	Rancid odor
16	0	yellowish brown	−	+	21.3	131.3	26.81	dark reddish brown	+-+
17	0.005	slight reddish brown	−	±	19.3	101.7	4.16	reddish brown	+
18	0.010	light reddish brown	−	−	23.6	95.1	5.15	light reddish brown	+
19	**0.025**	**light grayish brown**	**−**	**−**	**20.4**	**86.2**	**3.21**	**slight reddish brown**	**+**
20	0.050	dark grayish brown	∓	−	17.3	90.0	1.75	slight reddish brown	+

* a=Days after the treatment; b=Days after the becoming dry of sample.
Average temperature 4±5°C.
(−): No symptoms observed. (+): Development of symptoms.

Table 5. The conditions of salted-and-dried saury treated with **EP.**

No.	Concentration of EP in NaCl curing bath (%)	(Days of storage of samples)*								
		(a=6, b=1)			(a=15, b=10)					
		Color	Odor of EP	Taste of EP	Color	Racid odor	Rancid taste	Acid value	Peroxide value	Oxidized acid
21	0	yellow	−	−	reddish yellow	++	±	11.5	135.3	4.00
22	0.005	tinged with light red	−	−	reddish brown	+	±	10.3	51.1	−
23	0.010	tinged with dark red	−	−	yellowish brown	+·	±	11.8	125.8	3.85
24	**0.025**	**tinged with dark red**	**−**	**−**	**yellowish brown**	**±**	**±**	**11.7**	**67.7**	**3.55**
25	0.050	tinged with dark red	∓	∓	blackish brown	−	±	9.3	67.8	2.16

* a=Days after the treatment; b=Days after the becoming dry of sample.
Average temperature 12±5°C.
(−): No symptoms observed. (+): Development of symptoms.

Table 6. The conditions of salted-and-dried saury treated with **PL.**

No.	Concentration of PL in NaCl curing bath (%)	(Days of storage of samples)*										
		(a=6, b=1)			(a=23, b=18)					(a=28, b=22)		
		Color	Odor of PL	Taste of PL	Color	Odor of PL	Rancid odor	Taste of PL	Rancid taste	Acid value	Peroxide value	Oxidized acid
26	0	yellow	−	−	yellowish brown	−	+	−	+	11.6	132.4	16.15
27	0.005	light yellow	±	±	yellowish light brown	−	±	∓	±	11.1	117.5	6.31
28	**0.010**	**light yellow**	+	±	**yellowish light brown**	**−**	**−**	∓	±	**10.9**	**111.4**	....
29	0.025	pale yellow	+	+	yellowish light brown	±	−	+	−	9.7	127.6	4.19
30	0.050	pale yellow	++	++	yellowish light brown	+	−	++	−	12.4	153.6	7.81

* a=Days after the treatment; b=Days after the becoming dry of sample.
Average temperature 8±5°C.
(−): No symptoms observed. (+): Development of symptoms.

EP, or PL, when applied respectively to salted-and-dried saury in curing for 16 hours, was determined by preliminary test. The results were shown in Tables 1, 2, 3, 4, 5, and 6, respectively. A column shown in Gothic concerns the concentration judged as the marginal use.

It was obvious from these results, when 16 hours' curing was applied:

BHA: Marginal use should be 0.01%. When this antioxidant was used in a higher concentration a more protective effect was recognized; but the odor and taste specific to BHA were imparted to the products, making these products incopetent to be sold on the market. In addition this substance has a tendency of giving a blue or brown color to the products.

BHA'': Marginal use should be 0.01%. Beside the inferior efficacy due to the BHA itself, brown discoloration due to the PG,[33] a component of this antioxidant, spoiled the external appearance of the products. Therefore, even though the protective efficacy was rather strongly exhibited, the application of this substance at higher concentrations than those listed above would be unsuitable.

NDGA: Marginal use should be 0.025%. This substance did not impart its odor or taste to the marine products at all, but tended to impart a red color.

NDGA'': Marginal use shoud be 0.025% like to that of NDGA. The inferior efficacy of color development was slightly less in degree as compared with NDGA.

EP: Magrinal use should be 0.025%. The odor or taste of this antioxidant did not cause an inferior quality to the marine products in this experiment, but tended to cause somewhat stronger red color development.

PL: Marginal use is judged as 0.01%. A specific odor and taste of PL soaked deeply into the products, and in some products treated with 0.05% of this substance, the imparted unnaturalness was so serious that it cannot be eaten by any possibility. The external appearance of those which treated in a higher concentration of this substance seemed somewhat bleached.

(2) Comparison of relative efficacy of each antioxidant in their marginal use.[2]

According to the results obtained by preliminary experiments, samples of salted-and-dried saury were treated with abovementioned antioxidants at their marginal uses, respectively. The efficacies of the antioxidant as applied to these samples were compared with each other organoleptically as well as scientifically.

(a) *Results obtained by organoleptic evaluations.*

Color of the products: Change in color of each sample is shown in Table 7. In the first period of the storage, products applied with PL (No.37) were distinctly superior to others, and BHA-applied products (No. 32) were excellent in the second place. On the other hand, those treated with BHA'', NDGA, NDGA'', or EP, respectively, (Nos. 33, 34, 35, and 36), tended to discolor to red or brown. Generally, such developed color increasingly darkened with increasing storage time. But, the products applied with BHA or PL, respectively, kept an almost original fresh color for long period, their appearance being hence

Table 7. Color of salted-and-dried saury treated with antioxidant.

No.	Antioxidant		(Days of storage of samples)**				
	Kind	Concentration* (%)	(a=4, b=1)	(a=6, b=3)	(a=10, b=7)	(a=14, b=11)	(a=20, b=17)
31	Control	—	yellowish brown	brown	light reddish brown	light reddish brown	reddish brown
32	BHA	0.010	light yellowish brown	light yellowish brown	yellowish brown	yellowish brown	yellowish brown
33	BHA"	0.010	yellowish brown tinged with slight red	yellowish brown tinged with slight red	light brown tinged with light red	light brown tinged with red light	light brown tinged with black
34	NDGA	0.025	yellowish light brown tinged with light red	light brown tinged with red	light brown with tinged red	light brown tinged with slight black	light brown tinged with black
35	NDGA"	0.025	yellowish brown tinged with light red	light brown tinged with red	light brown tinged with red	light brown tinged with slight black	light brown tinged with black
36	EP	0.025	yellowish brown tinged with light red	light brown tinged with red	light reddish brown	light reddish brown	reddish brown
37	PL	0.010	light yellow	light yellowish brown	yellowish brown	light reddish brown	reddish brown

* Concentration in NaCl solution (marginal use)

** a=Days after the treatment; b=Days after the becoming dry of sample.
Average temperature 12 ± 5°C.

superior to that of control. After 20 days of storage, only the products treated with BHA were found keeping the original color and judged to be by far superior to others. Those treated with EP or PL were equal to control and distinctly superior to those treated with BHA'', NDGA, or NDGA'', which were tinged more heavily with black than control.

Odor of the products: Change in odor of each sample is shown in Table 8. Even in the first period of storage, control and others treated with EP (No. 36), and PL (No. 37), already developed rancid odors to a slight extent, and with the progress of storage these inferior odor increased. Those treated with BHA, BHA'', NDGA, or NDGA'' (Nos. 32, 33, 34, and 35) were quite free from rancid odors at the end of 20 days storage.

Table 8. Development of rancid odor in salted-and-dried saury treated with antioxidant

No.	Antioxidant		(Days of storage of samples)**				
	Kind	Amount* (%)	(a=4,b=1)	(a=6,b=3)	(a=10,b=7)	(a=14,b=11)	(a=20,b=17)
31	Control	–	+	+	++	++	++
32	BHA	0.010	–	–	–	–	∓
33	BHA''	0.010	–	–	–	–	–
34	NDGA	0.025	–	–	–	–	–
35	NDGA''	0.025	–	–	–	–	–
36	EP	0.025	∓	∓	+	+	+
37	PL	0.010	+	+	+	++	++

* Concentration in NaCl solution (marginal use).
** a=Days after the treatment; b=Days after the becoming dry of sample.
(–): No symptoms observed. (+): Development of rancidity.
Average temperature 12±5°C

Table 9. Peroxide value* of salted-and-dried saury treated with antioxidant.

No.	Antioxidant		(Days of storage of samples)***				
	Kind	Amount** (%)	(a–4,b–1)	(a–6,b–3)	(a–10,b–7)	(a–14,b–11)	(a–20,b–17)
31	Control	–	66.1	126.2	156.5	173.6	240.2
32	BHA	0.010	21.0	20.9	28.6	25.1	26.8
33	BHA''	0.010	8.2	22.8	31.9	17.8	20.1
34	NDGA	0.025	9.7	23.8	19.8	35.6	38.8
35	NDGA''	0.025	9.3	48.2	21.7	63.6	46.9
36	EP	0.025	14.6	87.0	125.5	114.6	140.9
37	PL	0.010	76.8	74.6	166.6	120.6	155.7

* expressed as milliequivalent /kg. of oil.
** Concentration in NaCl solution (marginal use).
*** a=Days after the treatment; b=Days after the becoming dry of sample.
Average temperature 12±5°C.

(b) *Results by chemical evaluations.*

i) *The sample stored in original form in the dark.*

Peroxide value of the oil extracted from the fish samples stored in original form in a dark place was determined. The results are shown in Table 9.

It is obvious from the changes in peroxide value shown in Table 9 that the different kinds of antioxidants employed differ apparently from one another also with regard to their efficacy. This coincides quite well with the organoleptic result obtained. In the case of control sample wherein the use of antioxidant was dispensed with, accumulation of peroxide increased abruptly, while the samples treated with antioxidants showed a comparatively low value of peroxide, the peroxide, in turn, accumulating gradually in a long period of storage. Especially the products treated with either BHA or BHA'' showed the same results having a noticeably low value during the whole period of this experiments. Those treated with NDGA or NDGA'' were excellent, comming the next to the products applied with BHA or its mixture, BHA'', while those treated with EP or PL showed a slightly lower value in comparsion with control.

ii) *The sample stored in a comminuted state.*

In order to reaffirm the difference between the efficacies of indivual antioxidants employed, the authors carried out another series of examination wherein elimination of sampling error and acceleration of oxidation were realized by using the samples enlarged in surface. Fish samples as soon as they became dry were comminuted with chopper and subjected to the test of preservability. Changes in peroxide value and acid value measured for the oils extracted from these comminuted samples are shown in Tables 10 and 11.

Table 10. Peroxide value* of oil of salted-and-dried saury treated with antioxidant. (The fish flesh was comminuted with chopper before storage).

No.	Antioxidant		(Days of storage of samples)***			
	Kind	Amount** (%)	(a=4,b=1)	(a=6,b=3)	(a=8,b=5)	(a=10,b=7)
38	Control	–	86.3	213.8	301.6	468.9
39	BHA	0.010	20.1	44.7	60.3	107.3
40	BHA''	0.010	15.3	45.7	63.5	106.1
41	NDGA	0.025	42.0	128.5	212.2	403.9
42	NDGA''	0.025	32.3	107.8	230.2	411.2
43	EP	0.025	37.6	148.7	249.6	455.6
44	PL	0.010	55.7	179.8	287.2	533.1

* Expressed as milliequivatent /kg of oil.

** Concentration in NaCl solution (maginal use).

*** a=Days after the treatment; b=Days after the becoming dry of sample. Average temperature 12±5°C.

When the peroxide values of extracted oil are compared between the products stored in original form (i) and those in comminuted form (ii), the changes

in the latter can be said to take place more rapidly than in the former and the differences in the effect between various antioxidants appear more distinctly. The protective efficacy of antioxidant is recognized for every case already in the first period of storage, but with the progress of storage, the samples except those treated with BHA or BHA'' show rapidly increasing peroxide values which become sooner or later coincident with those of controls. In those treated with NDGA or NDGA'', the efficacy of antioxidant is somewhat lasting, but in those treated with EP or PL the efficacy of antioxidant is hardly maintained.

Table 11. Acid value of oil of salted-and-dried saury treated with antioxidant. (The fish flesh was comminuted with chopper before storage).

No.	Antioxidant		(Days of storage of samples)**			
	Kind	Amount*	(a=4,b=1)	(a=6,b=3)	(a=8,b=5)	(a=10,b=7)
		(%)				
38	Control	–	18.2	18.8	19.9	22.8
39	BHA	0.010	12.2	12.5	13.5	15.3
40	BHA''	0.010	15.5	16.3	17.0	17.2
41	NDGA	0.025	15.6	15.8	18.0	18.7
42	NDGA''	0.025	12.0	12.5	13.5	15.3
43	EP	0.025	14.5	16.1	17.7	18.9
44	PL	0.010	16.3	16.6	18.8	19.5

* Concentration in NaCl solution (marginal use).
** a=Days after the treatment; b=Days after the becoming dry of sample. Average temperature 12±5°C.

The acid value estimated (Table 11) shows a situation almost similar to those suggested by peroxide value. Taking a general view, treatment with BHA or NDGA'' keeps a comparatively low value, that with BHA'', NDGA or EP comes next and that with PL produces little difference distinguishable from the control.

II. Salted salmon.

(1) **Kairyo-aramaki** (ordinary type of salted salmon).

(a) *Applicability of antioxidant.*[3]

The results obtained for the *Kairyo-aramaki* treated with BHA, EP, and PG, respectively , are shown in Tables 12, 13, 14, and 15.

It may be judged from these results, that each antioxidant employed was more or less effective in every case. EP and PG, however, may not be recognized as satisfactory because these two antioxidants showed a tendency of imparting a violet color to fish flesh and external surface of the products. This unsatisfactory result forced the authors to inquire into the marginal use of BHA and to repeat a similar experiment but with only BHA.

(b) *Determination of marginal use of BHA.*[4]

The results are shown in Tables 16 and 17.

Table 12. External appearance of the processed salmon. (*Kairyo-aramaki*)

No.	Antioxidant		After 3 months' storage		After 6 months' storage	
	Kind	Concentration* (%)	a**	b***	a**	b***
45	Control	—	normal	normal	normal, without luster	tinged with yellow
46	BHA	0.006	normal	normal	normal' slight	tinged with light yellow
47	BHA	0.015	normal	normal	lustrous normal, lustrous	tinged with slight yellow
48	EP	0.030	tinged with slight violet	tinged with reddish violet	normal, lustrous	tinged with slight reddish violet
49	PG	0.030	tinged with slight violet	tinged with reddish violet	normal, lustrous	tinged with slight violet

* The concentration referred to fish flesh which was calculated from the quantity of salt (30% of the fish) employed in this work.

** Body

*** Part of ventral median line.

Stored in a temperature range of –5° to 0°C.

Table 13. Color of the processed salmon flesh (*Kairro-aramaki*)

No.	Antioxidant		After 6 months' storage		After 6 months' storage		
	Kind	Concentration*	Part (a)**	Part (b)	Part (a')	Part (a'')	Part (b)
45	Control	—	light red	light yellow	light yellow	slight yellow	light yellow
46	BHA	0.006	light red, looks fresh	tinged with light red	light yellow	light red, looks fresh	light yellow
47	BHA	0.015	light red, looks fresh	tinged with light red	light red	light red, looks fresh	tinged with light red
48	EP	0.030	light red, tinged with violet, looks fresh	reddish violet	light red, tinged with light violet	light red tinged with light violet, looks fresh	dark violet
49	PG	0.030	light red, looks fresh	reddish violet	tinged with light red	light red, looks fresh	tinged with slightly violet

* The ratio to fish flesh calculated from the quantity of salt (30% of the fish) employed in this work.

** Part (a) = parts 1, 2, 3, 4, shown in Fig. 1.
Part (b) = parts 1', 2', 3', shown in Fig. 1.
Part (a') = part (a) dressed after 3 months.
Part (a'') = part (a) dressed after 6 months.

Stored in a temperature range of –5° to 0°C.

Table 14. Odor of the processed salmon flesh. (*Kairyo-aramaki*)

No.	Antioxidant		After 3 months		After 6 months
	Kind	Concentration*	Odor of antioxidant	Rancid odor	Rancid odor
		(%)			
45	Control	–	–	–	++
46	BHA	0.006	–	–	+
47	BHA	0.015	–	–	±
48	EP	0.030	–	–	±
49	PG	0.030	–	–	±

* The ratio to fish flesh calculated from the quantity of salt (30% of the fish) employed in this work.
(–): No symptoms observed. (+): Development of rancidity.
Stored in a temperature range of –5° to 0°C.

Table 15. Peroxide value of the oil extracted from processed salmon (*Kairyo-aramaki*) after 6 months' storage. (meq./kg. of oil).

No.	Antioxidant		Peroxide value of extracted from			
	Kind	Concentration*	Part 1**	Part 2	Part 1'	Part 2'
		(%)				
45	Control	–	119.3	120.6	279.9	180.1
46	BHA	0.006	95.1	95.3	93.3	98.8
47	BHA	0.015	95.4	78.7	68.7	69.6
48	EP	0.030	167.5	131.4	185.3	105.9
49	PG	0.030	102.6	–	121.5	71.6

* The ratio to fish flesh calculated from the quantity of salt (30% of the fish) employed in this work.
** Shown in Fig. 1.
Stored in a temperature range of –5° to 0°C.

About 17 days after control began to yellow on the external parts of body and also to be reduced in redness of the flesh of abdominal cavity wand. With the progress of storage period, these symptoms of rancidity grow more and more apparent and so that the control became at last entirely spoiled by formation of brown spots on the body and also full of strong rancid odor. While every sample treated with BHA was kept in an almost good state during the whole storage period, the application of BHA even at only 0.005% (No. 51) was found sufficiently effective in protecting a sample from deterioration over 60 days.

Though the salted salmon treated with comparatively high concentration of BHA tended to yellow on the external parts, which may be attributed to a phenomenon somewhat different from that of rusting, such discoloration induced by BHA may be regarded as negligible, especially in the case of usage lower than 0.02% based upon the fish flesh, because the trifle defect of this agent is by far tolerable as compared with the serious harm which rancidity and rusting do.

Table 16. Organoleptically detected rancidity and rusting of salted salmon. (*Kairyo-aramaki*)

No.	Concentration of BHA to fish flesh*	Organoleptic decision of rancidity and rusting** (Days of storage)***				
		(0)	(17)	(31)	(46)	(60)
	(%)					
50	0	−	+	+	++	+++
51	0.005	−	−	±	±	+
52	0.010	−	−	±	±	±
53	0.020	−	−	±	±	±
54	0.040	−	−	±	±	±

* Ratio calculated from the quantity of salt (40% of the fish) employed in this work.

** (−): The sample remained in their initial state.
(+): Development of rancidity and rusting.

*** Stored at room temperature of winter, 4° to 13°C.

For reference, these samples after 46 days storage taken on color film are presented in Figures 2 and 3.

In addition, the tendency of increasing peroxide value of the oil extracted from these products are parallel with the results obtained by organoleptic evaluations, and the effect of added BHA is distinctly recognised as such that can be seen in Table 17.

Table 17. Peroxide value (meq./kg of oil) of the oil extracted from processed salmon. (*Kairyo-aramaki*)

No.	Concentration of BHA to fish flesh*	Part**	Peroxide value of extracted oil (Days of storage)***				
			(0)	(17)	(31)	(46)	(60)
	(%)						
50	0	a	25.5	164.1	101.6	111.3	160.9
		b	44.4	97.7	146.6	109.2	181.6
51	0.005	a	15.6	33.2	64.8	55.7	99.9
		b	27.9	37.8	70.5	67.8	127.7
52	0.010	a	46.6	47.3	27.1	65.9	63.6
		b	20.7	31.0	33.1	59.7	45.4
53	0.020	a	19.8	34.0	57.6	45.4	45.8
		b	24.7	46.0	29.4	30.0	31.7
54	0.040	a	37.3	47.5	65.9	49.1	47.6
		b	56.5	41.2	62.9	31.1	52.6

* Ratio calculated from the quantity of salt (40% of the fish) employed in this work.

** a = Abdominal part, 2' and 3' shown in Fig. 1.
b = Body, 2 and 3 shown in the same place.

*** Stored at room temperature of winter, 4° to 13°C.

(2) **Aramaki** (mildly salted salmon).[5]

The antioxidants BHA, BHT, and IAG'' were applied to *Aramaki*, respec-

tively, in the quantities shown in Table 18, and their protective efficacy of protecting the products from suffering rancidity and rusting were evaluated.

Table 18. Dosages of antioxidants referred to common salt and to fish flesh (the values are common for BHA, BHT, and IAG".). (*Aramaki*)

No.	Antioxidant per cent.	
	In common salt	In fish flesh
55	0	0
56	0.004 (1/25000)	0.00125 (1/80000)
57	0.008 (1/12500)	0.0025 (1/40000)
58	0.016 (1/6250)	0.005 (1/20000)
59	0.032 (1/3125)	0.01 (1/10000)
60	0.064 (1/1563)	0.02 (1/5000)

(a) *Condition of each batch after 3 months' storage.*

Control batch: Owing to the storage in better conditions than the case of *Kairyo-aramaki*, no symptoms of rancidity and rusting were observed. Peroxide value of extraced oil from abdominal parts (Parts 2' and 3' shown in Fig. 1) of the products were 24 to 52 (meq./kg. of oil).

BHT-treated batch: No symptoms of rancidity and rusting were observed. All samples were recognized neither as discolored nor as badly changed in organoleptic aspect in virtue of the BHT added. Peroxide values of oil extracted from abdominal part of the products were in the range of 17 to 32 (meq./kg. of oil).

BHA-treated batch: No symptoms of rancidity and rusting were observed. However, the products treated with comparatively high concentration of BHA, such as 0.005% to 0.02% relative to the fish flesh, were degraded in external appearance as a result of acquiring a slight yellow color. Perhaps these yellowish discolorations will be the similar to those observed in the case of *Kairyo-aramaki* applied with an excess of BHA. Peroxide values of the oil extracted from abdominal part of the products were in the range of 11 to 31 (meq./kg. of oil).

IAG"-treated batch: No symptoms of rancidity and rusting were observed. As IAG" contains ester of gallic acid as a main constituent, occurrence of discoloration of product was feared prior to this experiment from the results obtained in the case of PG or EP. Just as expected, a tendency of violet discoloration on the abdominal cavity wand as well as in abdominal parts was recognized. Therefore, when more than 0.01% of this substance is used, market value of the products is supposed to be greatly depreciated. Peroxide values of oil extracted from abdominal part were in the range of 13 to 32 (meq./kg. of oil).

(b) *Condition of each batch after 6 months' storage.*

Control batch: Every product of this batch showed a definit symptoms of rancidity and rusting. Yellowish discoloration at abdominal part, faded pink color at abdominal cavity wand, and a tendency of discoloration to yellowish or brownish color at tail fin were observed with most of the products. Peroxide

values of oil extracted from abdominal part were in a range of 138 to 190 (meq./kg. of oil).

BHT-treated batch: No symptoms of rancidity and rusting were observed. Such phenomena as discoloration in external appearance, reduction of pink color of abdominal cavity wand and decrease of the luster of the epidermis were not recognized. Peroxide values of oil extracted from these products were in the range of 26 to 35 (meq./kg. of oil), and no significant difference was detectable compared with those measured 3 months before.

BHA-treated batch: With the exception of a slight fading of pink color on abdominal cavity wand, no symptoms of rancidity and rusting were observed. The results were generally good. But yellowish discoloration in abdominal part, which was probably due to the BHA, was observed to a slight extent in the applications of relatively high concentrations such as 0.005% and 0.01%. This kind of discoloration was remarkable in the case of 0.02%. Peroxide values of extracted oil were 31 to 78 (meq./kg. of oil) and somewhat increased compared with those determined 3 months before.

IAG "-treated batch: Although both discoloration in external appearance and fading color on abdominal cavity wand, which were due to rancidity and rusting, were observed at lower concentrations of IAG" but with increasing concentration these inferior symptoms became slighter, clearly showing the efficacy of antioxidant. Discoloration which presumably due to IAG " itself decreased somewhat as compared with that observed before 3 months, except that in the case of high concentration (over 0.01% based on the weight of fish) the same states was observed as that of 3 months before. For the sample treated with relatively low concentrations of IAG" in which the discoloration was negligible, observed peroxide value was as low as 57 (meq./kg. of oil) when 0.005% (based on the weight of fish) of this substance was applied; and for other samples treated with smaller concentrations the value was the same as that of control.

(3) Influence of common salt to antioxidants.[4)5)]

When the calculated amount of various antioxidants was mixed respectively with the common salt and stored in room temperature, no changes were observed in the case of BHA, but the salt added with EP or PG tended to develop a light brown or yellowish brown color. Such discoloration seems to be caused by the interaction between antioxidants and iron inpurity[3)] in the common salt employed. These phenomena are shown in Figure 4 by the help of color film.

Since it had been said that the common salt mixed with BHT tended to develop yellow color, the salt was mixed with BHT in the range 0.01 to 0.40% and stored in room temprature. After 1 month's storage no changes was observed, but, after 2 months' storage, the salt added with BHT over 0.10% developed a yellow color and both with 0.20% and 0.40% said kind of discoloration became more apparent.

Discussion

I. Applicability of an antioxidant to marine products.

(1) Salted-and-dried saury.

From the results presented above the following conclusion may be derived about the efficacy of each antioxidant examined.

BHA is most effective in the range which gives no bad effects on the products, and also most excellent among the antioxidants so far examined, so it may be considered as an antioxidant fitted for protecting salted-and-dried fish from suffering the rancidity and rusting.

BHA" showed an excellent protecting efficacy equal to those of BHA, and in a few cases, though only in a few degree, this substance was rather superior to BHA. However, it was found that BHA" is not very satisfactory because of its tendency of causing red color development, though slightly, even at comparatively low concentrations in application. This discoloration is likely due to the interaction of PG component in the antioxidant and the iron in fish or in salt. Owing to this inferiority, BHA" is to be looked upon as inferior to BHA.

NDGA and **NDGA"** showed a good protective efficacy, ranking next to above two antioxidants, BHA and BHA". But these two antioxidants tended to cause red color development on the fish products, and cannot be recommended to be applied to salted-and-dried fish. Considered from the chemical structure of NDGA, this color development may certainly be due to the interaction between NDGA and the iron in fish flesh or in salt as in the case of PG.

EP was found to be inferior in efficacy as compared with BHA, BHA", NDGA, and NDGA". and could not maintain its efficacy beyond the earliest period of storage. Moreover, since this substance tended to produce red coloration, its application is not recommendable in the case of salted-and-dried fish.

PL showed no positive effect in the prevention of rancidity and rusting. The efficacy of this substance, despite of its having been thought theretofore as an effective retardant against rancidity and rusting of marine products[6)9)] turned out much inferior to the other antioxidants applied under the same condition. This demonstrates the outstandingly strong efficacies of synthetic antioxidants.

(2) Salted salmon.

PG and **EP** were regognized as inapplicable to salted salmon, owing to their drawback of giving undesirable color to the products. This discoloration occurred not only in fish flesh but also in the salt which had been mixed with antioxidants and stored out of contact with fish. One of the sources which led to the discoloration of EP or PG applied *Kairyo-aramaki*, is attributable to the common salt used itself. Accordingly, in the case of applying such antioxidants as EP and PG to salting the fish, it is necessary for dry salting to carefully select a common salt of suitable grade.

BHA exhibited an excellent efficacy in protecting *Kairyo-aramaki* from suffering rancidity and rusting. When this substance was added to the salt and was used in first salting (40% of fish weight) of 10 days, good results were obtained

in general. Its usage in the range of 0.005% to 0.04% (by the fish weight), and especially at 0.01%, was recognizaed to be most satisfactory, because the discloration due to BHA itself proved to give practically no trouble. For *Aramaki*, even 0.0025% use of BHA exhibited a sufficiently good effect.

The discoloration developed in the salted salmon in high concentration application of BHA (which gives a yellow color to the fish body) will be a problem to be resolved in future.

BHT scarcely caused *Aramaki* to be discolored in the scope of this experiment, and the effect of this substance became somewhat better with the increase of the using quantity. In general, the effect was superior to that of BHA in the case of *Aramaki*. Concentrations in the range of 0.0025% to 0.005% by weight of fish will be sufficient.

BHT added salt developes yellow color as mentioned above. Though more investigation must be done about the cause and mechanism of this color development, 0.02% BHT to salt is sufficient in practice. With such low concentration no trouble of discoloration is given rise to. So, there is obviously no trouble if BHT and salt are uniformly mixed with care.

IAG " could not exhibit any protective efficacy when applied to the *Aramaki* in such a low concentration as causes no discoloration. Perhaps it will give analogous results in the case of *Kairyo-aramaki*.

(3) Situation of experimental marginal use to legally approved amount of antioxidant.

In the course of this work, the Welfare Ministry of Japan has permitted the usage of some antioxidants for marine products in the realm of a condition limited from the hygienic point of view[31].

In our experiment abovementioned, both BHA and BHT have been revealed as most powerful antioxidants effective for protecting marine products from rancidity and rusting. Their legally approved limit, on the other hand, is 0.2 gram per 1 kg. of dried fish and salted fish, respectively.

From the results of present experiments, for salted-and-dried saury BHA exhibited a sufficitent effectiveness with about a half of the approved quantity, and for *Kairyo-aramaki* and *Aramaki* with an amount less than the approved one, i. e., 0.1 g. and 0.0025 g. per 1 kg. of raw fish, respectively. BHT showed a good effect even at an amount of 0.025 to 0.05 g per 1 kg. of raw fish. Accordingly there produces no problem in using these antioxidants to marine products in their marginal uses.

II. Efficacy of antioxidants in evading the rancidity and rusting of marine products.

In order that the efficacy of an antioxidant added to marine products may be exhibited as fully as possible, it will surely be necessary that the antioxidant is sufficiently soluble in the oil of fish body. Then, it must be taken into account the nature of the antioxidant to be applied to the marine

products, especially solubility of the antioxidant in oil.

For instance, while the efficacy of NDGA is reported as superior to that of BHA in direct application to lard or cottonseed oil[35], the former showed a very weak effect when applied to salted-and-dried saury. The difference have certainly arisen from the inferiority of NDGA to BHA in the solubility in oil[36] and hence from the corresponding insufficient permeation into fish oil. One of the reason for the fact that BHA behaved as the most efficient inhibitor against the rancidity and rusting of marine products among several antioxidants so far examined may possibly be attributed to its excellent solubility in oil.

The same conclusion should be drawn about the effect of citric acid available as synergist for antioxidant. In the case of oil or fat, it is well known that when citric acid is used as added with BHA or NDGA[37], the effect of these antioxidant is emphasized to a noticeable extent. In the case of salted-and-dried saury, however, such a phenomenon has not been proved to be true. This may be considered to be due to the low solubility of citric acid in oil, which will hardly produce a good permeation into fish oil.

In general, it is likely that the higher the solubility of antioxidant in oil, the shorter the time required in dissolution of the antioxidant. With a less soluble antioxidant a sufficient permeation and antioxidative effect can hardly become apparent, except when it is kept in contact with the fish for a long time. Despite of its low solubility in oil[40], BHT is an excellent one among many antioxidants. This substance is less effective than BHA, when applied to salted-and-dried saury and *Kairyo-aramaki*, in the case of which the time for which the antioxidant and the fish are kept in mutual contact is comparatively short. But, in the case of *Aramaki*, in the case of which a long contact is possible, good results are truly obtained with BHT as described above. These facts support our view that the protective efficacy of an antioxidant for marine products is affected to a considerable rate by the solubility of the antioxidant in oil.

Another important factor which makes BHA surpass NDGA in preventing marine products from their suffering the rancidity and rusting is the effect of free fatty acids present in these products. Olcott[36] investigated the antioxidant effectiveness in unsaturated oils, and cleared that it is remarkably influenced by the presence of free fatty acids. His study is concerned with the relative effectiveness of antioxidants in trilinolein. He found that the oxidation induction period observed whith NDGA and BHA in the absence of free fatty acid were 29 and 8 days, but in the presence of 1% linoleic acid were 5.0 and 3.0 days, and with 2.5%, 1.5 and 3.0 days, respectively. The amount of free acids present in the oil of salted-and-dried saury just finished of drying was as large as about 6 to 9% when calculated from Table 11 as oleic acid. This suggests that the effect of free acid can be expected to be of considerable strength.

As another reason of the superiority of BHA to NDGA in the protection of marine products, it is possible to consider that BHA might have a specific effect in protecting the oxidation of polyunsaturated fatty acids. Olcott, however, stated that neither the degree of unsaturation of fatty acid ester nor the type of ester linkage had no relation to the superiority of NDGA to EMQ (6-ethoxy-2,2,4-trimethyl-1, 2-dihydroquinoline. *Santoquin*) in the same investigation[36]. His results

scarcely throw any positive suggestion on this assumption. Accordingly, only from a further investigation the solution of this problem may be expected.

Summary

In the aspect of protecting some kinds of marine products from deterioration due to rancidity and rusting in the case of storage at ordinary temperature and in the presence of air, several antioxidants were examined of their applicability to salted-and-dried fish and salted fish. Each one of antioxidants, i. e., butyl hydroxyanisole (BHA), nordihydroguaiaretic acid (NDGA), propyl gallate (PG), isoamylgallate (IAG), ethyl protocatechuate (EP), dibutyl-p-hydroxytoluene (BHT), and pix liquida (PL) was tested by applying it alone or in combination with citric acid to the raw fish in the form of a curing solution or as mixture with salt. Evaluation of the applicability of these antioxidants to said marine products led to the following results:

(1) A deterioration of marine products due to the oxidation of their oil, namely rancidity and rusting, can be evaded to considerable extents by the use of proper antioxidant in a proper way. BHA showed an excellent protective efficacy in both the cases of salted-and-dried fish and salted fish, while the effect of NDGA was found far inferior. This is considered as due to the difference of solubility in oil between these two antioxidants.

(2) Among all the antioxidants examined, gallic acid esters and allied compounds were recognized as unsatisfactory, because they reacted with iron existing in the fish flesh or in the salt and yielded colored material which spoiled the external appearance of the products.

(3) The authors consider that in order to make a poorly oil soluble antioxidant fully exert its efficacy, it is necessary to keep the antioxidant in contact with the raw fish for a long time so as to accomplish a sufficient permeation of antioxidant into fish oil. Thus, BHA, when applied to *Aramaki*, brought excellent results as expected.

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Fig. 2

Fig. 3

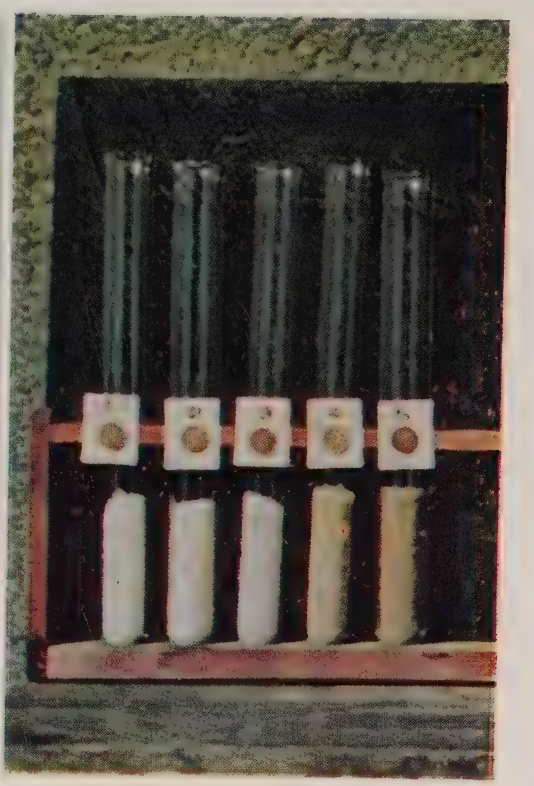

Fig. 4

PLATE XIX

Fig. 2. Protective efficacy of BHA at various concentrations discernible on the external appearance of salted salmon (*Kairyo-aramaki*) after 46 days' storage. Samples correspond to Nos. 50, 54, 53, 52, and 51, respectively, from top to bottom. (Fuji color reversal, f 8, 1/50, Leica IIIC, Summitar F 2.)

Fig. 3. Protective efficacy of BHA at various concentrations discernible on the color of fish flesh of salted salmon (*Kairyo-aramaki*) after 46 days' storage. Samples correspond to Nos. 50, 54, 53, 52, and 51, respectively, from top to bottom. (Fuji color reversal, f 8, 1/50, Leica IIIC, Summitar F 2.)

Fig. 4. Change in external appearance of common salt added with anti oxidant. No 1. Control; No. 2, BHA 0.02%; No. 3, BHA 0.05%; No. 4, EP 0.10%; and No. 5, PG 0.10%. (Fuji color reversal, f 8, 1/100, Konica IIIA, Hexanone F 2.).

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STUDY ON THE DISPOSITION OF FISH TOWARDS LIGHT (NO. 5.) THE STRENGTH OF ILLUMINATION COMFORTABLE TO ENGRAULIS JAPONICUS

Yutaka Imamura and Shoichi Takeuchi

(Received Feb. 15, 1960)

One of the authors has already reported on the behaviors of a few kinds of fishes separately observed in an experimental tank provided with a light source variable in strength of illumination. According to his observation, carp (*Cyprins Carpio Linne*), mackrel (*Scomber Japonicus*), horse-mackerel (*Trachrous Japonicus*) and saury pike (*Cololabis Saira*) equally have a habit of gathering by preference into a quiet group in a dim place from which a few indefinite members of the group occasionally visit a lighter place. A term designated by the author as 'gathering ratio of fish' or 'fish gathering ratio' has been proposed as a measure for numerically expressing the distribution pattern of fish in the tank. The value of this term was found to be governed, so far as the previous expriments indicate, almost exclusively by the strength of the illumination prevailing in the tank, as seen from the results of observation which are briefly restated in the following.

'Fish gathering ratio' obtained in the previous experiments.

Carp: 75% in a place of 18–49 *lux* in a tank kept exposed to an illumination graded from 18 to 4100 *lux*.

91% in a place of 0.2–5 *lux* in a tank kept exposed to an illumination graded from 0.2 to 110 *lux*.

69% in a place of 2.5–32 *lux* in a tank kept exposed to an illumination graded from 2.5–950 *lux*.

Macherel: 64.6% in a place of 2.5–2.8 *lux* in a tank kept exposed to an illumination graded from 2.5 to 39.5 *lux*.

Horse-macherel: 64.2% in a place of 2.5-2.8 *lux* in a tank kept exposed to an illumination graded from 2.5 to 39.5 *lux*.

In a field survey, saury pike was found to behave to light almost similarly to the above three kinds of fishes.

Now, the above reviewed tendency of fishes that they assemble by preference in a dim place is taken to all appearance to be contradictory to the general knowledge about the merit of utilizing a fish-luring light. Since the strength of light that finds favor with a fish, however, may still be possible to vary according as the fish differs in species, the authors dared in this time to repeat a few experiments similar to those already reported but with a sort of anchovy (*Engaulis Japonica*) as test fish which is known as one of the fishes caught habitually by making good use of a fish-luring light, and tried to ascertain the said possibility.

Arranegements for experiment

A concreate tank with a length of 4.8 m, a breadth of 1.5 m and a height of 0.75 m was employed as experimental tank. This was filled with water up to 60 cm from the bottom. Referring to white lines drawn on the surface of the bottom the inner space of the tank was divided into 27 sections equally having

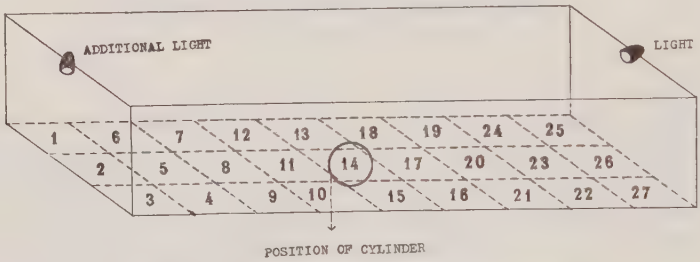

Fig. 1. Arrangement for the experiment.

a rectangular base of 50 cm^2. A reflecter white lamp of 300 watt as light source was set as illustrated in Figure 1. The light was projected at an angle of 45 degree to water surface. In case of need, a white lamp of 20 watt was used in addition to said 300 watt lamp. The strength of illumination, which was regulated by means of a slidax so that the voltage applied to the lamps might be varied at need so as to be 30 volt, 60 volt and 100 volt, respectively, was measured by exposing an under water luxmeter to the light reaching the central part of each sectional area prepared as above on the tank bottom. Two shadowed domains were also prepared at need with appropriate boards in water in places voluntarily chosen in tank. As test fish 500 individuals of anchovy (*Engraulis Japonica*) were provided, which had been bred in a crawl for ten days in Tateyama Bay prior to subjecting them to an experiment. The body length and the body weight of these individuals were 10.1 cm and 8.47 g, respectively, on an average. A temperature of 18.9 -9°C was applied to the water in the tank. Five men participated together as operators in carrying out the present experiment.

Process of experiment

1. A celluloid cylinder 50 cm in diameter and 70 cm in length was stood upright in a specified place in the tank and the test fish were kept in this cylinder for ten minutes so as to be previously adapted to dark.
2. The cylinder was then removed out of the tank with simultaneous putting on the light and setting the fish free.
3. Observing the behavior of the fish, the pattern of fish distribution in the tank was recorded by counting their individuals found in each section at intervals of five minutes. Such obervation as this were continued for one hour and repeated two times in any one of the experiments carried out at different strengths of illumination. Individuals of test fish, the number of which amounted in a run to 27, were renewed after every run. All the ex-

periments were performed in complete absence of any bait so that the fish behavior observed might be regarded as that which was conducted purely by phototaxis of the fish.

Preliminary experiment

Table 1. The pattern of the distribution of fish in the preliminary experiment

Section number	Number of fish		Correction term	Section number	Number of fish		Correction term
	observed	averaged to a section			observed	averaged to a section	
1	14	24	+ 10	15	41	24	− 17
2	23	〃	+ 1	16	34	〃	− 10
3	22	〃	+ 2	17	17	〃	+ 7
4	48	〃	− 24	18	8	〃	+ 16
5	38	〃	− 14	19	11	〃	+ 13
6	15	〃	+ 9	20	25	〃	− 1
7	11	〃	+ 13	21	31	〃	− 7
8	38	〃	− 14	22	40	〃	− 16
9	31	〃	− 7	23	42	〃	− 18
10	36	〃	− 12	24	11	〃	+ 13
11	17	〃	+ 7	25	13	〃	+ 11
12	13	〃	+ 11	26	18	〃	+ 6
13	8	〃	+ 16	27	22	〃	+ 2
14	21	〃	+ 3	In total	648	648	0

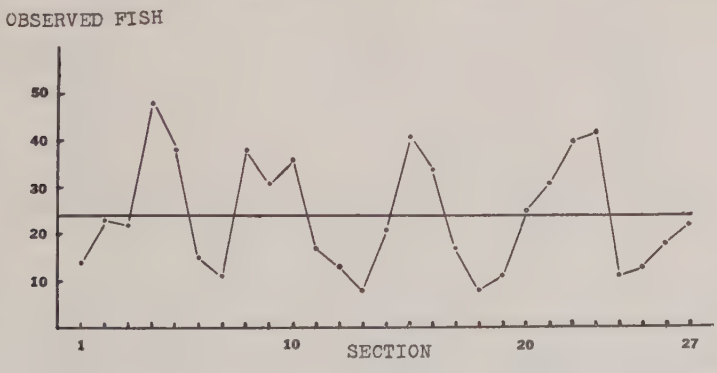

Fig. 2. The pattern of the distribution of fish (Preliminary experiment)

A preliminary experiment was carried out in a dark room, the lightness in which was measured all over to be 0 lux. It was examined how fish distribution was affected by the factors: dimension of the tank used, the nature of tank wall and the position in which the celluloid cylinder had been set. In this preliminary experiment, fish distribution was inferred from the results of counting the fish individuals observed in each section of the tank using momentary light-

Table 2. The pattern of the distribution of fish in Experiment (No. 1.)

Section number	Values of illumination		Number of fish	Correction term	Corrected values	Corrected values adjusted for comparison
	lux	Rank				
1	0.01	1	0	+ 10	10	23
2	0.02	2	51	+ 1	52	65
3	0.03	3	51	+ 2	53	66
4	0.03	3	51	− 24	27	40
5	0.03	3	62	− 14	48	61
6	0.01	1	4	+ 9	13	26
7	0.01	1	18	+ 13	31	44
8	0.02	2	50	− 14	36	49
9	0.03	3	27	− 7	20	33
10	0.03	3	66	− 12	54	67
11	0.02	2	25	+ 7	32	45
12	0.01	1	12	+ 11	23	36
13	0.01	1	2	+ 16	18	31
14	0.02	2	7	+ 3	10	23
15	0.03	3	32	− 17	15	28
16	0.03	3	20	− 10	10	23
17	0.04	4	7	+ 7	14	27
18	0.01	1	3	+ 16	19	32
19	0.01	1	0	+ 13	13	26
20	0.03	3	25	− 1	24	37
21	0.03	3	11	− 7	4	17
22	0.03	3	3	− 16	*−13	0
23	0.04	4	114	− 18	96	109
24	0.01	1	3	+ 13	16	29
25	0.01	1	1	+ 11	12	25
26	0.02	2	3	+ 6	9	22
27	0.02	2	0	+ 2	2	15
In total			648	0	648	999

enings at intervals of five minutes (the durations of lightenings were 7–10 seconds). Correction terms for the errors arising from said factors were determined from the distribution thus inferred, and are given in Table 1 and Figure 1.

Main experiment No. 1

This experiment was performed on the 20th. of October in 1959 during a time extending from 1 p.m. to 3 p.m. using a natural light alone, namely, under completely excluding any artificial light from the tank. Every new 27 individuals of fish was used in every run. Similar observations were repeated two times. The results are summarized in Table 2 and Figure 2.

Table 3. The pattern of the distribution of fish in Experiment (No. 2.)

Section number	Values of illumination		Number of fish	Correction term	Corrected values	Corrected values adjusted for comparison
	lux	Rank				
1	0.10	1	45	+ 10	55	67
2	0.31	2	50	+ 1	51	63
3	0.10	1	16	+ 2	18	30
4	0.05	1	103	− 24	79	91
5	0.10	1	47	− 14	33	45
6	0.05	1	36	+ 9	45	57
7	0.04	1	4	+ 13	17	29
8	0.07	1	59	− 14	45	57
9	0.03	1	93	− 7	86	98
10	0.05	1	0	− 12	*−12	0
11	0.10	1	11	+ 7	18	30
12	0.10	1	17	+ 11	28	40
13	0.10	1	0	+ 16	16	28
14	0.10	1	10	+ 3	13	25
15	0.10	1	16	− 17	− 1	11
16	0.50	2	15	− 10	5	17
17	0.50	2	4	+ 7	11	23
18	0.30	2	0	+ 16	16	28
19	1.05	3	20	+ 13	33	45
20	1.65	3	17	− 1	16	28
21	1.23	3	3	− 7	− 4	8
22	3.50	4	4	− 16	*−12	0
23	6.60	5	60	− 18	42	54
24	3.40	4	4	+ 13	17	29
25	3.45	4	0	+ 11	11	23
26	8.50	5	12	+ 6	18	30
27	3.90	4	2	+ 2	4	16
In total			648	0	648	972

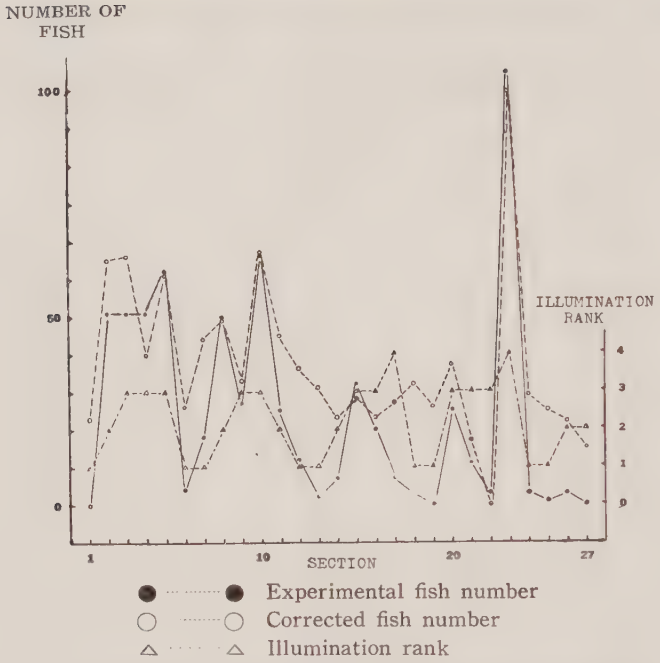

Fig. 3. The pattern of the distribution of fish (Experiment No. 1)

Main experiment No. 2

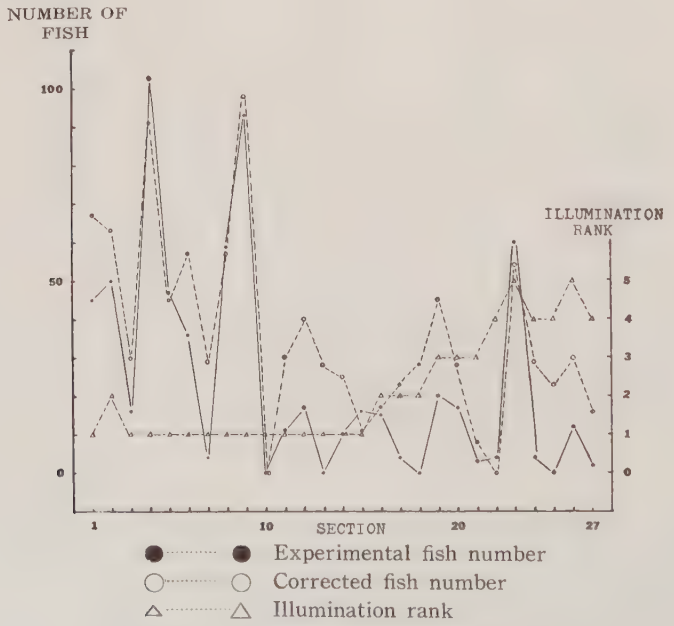

Fig. 4. The pattern of the distribution of fish (Experiment No. 2)

Table 4. The pattern of the disposition of fish in Experiment (No. 3.)

Section number	Values of illumination		Number of fish	Correction term	Corrected values	Corrected values adjusted for comparison
	lux	Rank				
1	0.20	1	50	+ 10	60	77
2	0.20	1	280	+ 1	281	298
3	0.20	1	117	+ 2	119	136
4	0.20	1	64	− 24	40	57
5	0.20	1	41	− 14	27	44
6	0.15	1	11	+ 9	20	37
7	0.22	1	1	+ 13	14	31
8	0.26	1	10	− 14	− 4	13
9	0.25	1	16	− 7	9	26
10	0.41	2	0	− 12	−12	5
11	0.40	2	3	+ 7	10	27
12	0.39	2	0	+ 11	11	28
13	0.30	2	0	+ 16	16	33
14	0.27	1	18	+ 3	21	38
15	0.30	2	0	− 17	*−17	0
16	2.60	3	0	− 10	−10	7
17	3.42	3	0	+ 7	7	24
18	2.60	3	5	+ 16	21	38
19	10.50	4	1	+ 13	14	31
20	17.60	4	1	− 1	0	17
21	10.10	4	0	− 7	− 7	10
22	23.50	4	1	− 16	−15	2
23	39.00	5	28	− 18	10	27
24	21.30	4	1	+ 13	14	31
25	26.50	4	0	+ 11	11	28
26	38.10	5	0	+ 6	6	23
27	35.00	5	0	+ 2	2	19
In total			648	0	648	1.107

Main experiment No. 2

In this experiment, a reflector white lamp of 300 watt as a main light source and another white lamp of 20 watt as an additional light source were employed both at 30 volt. This complementary lamp was set in such a manner that the light beam coming from which might be projected at an angle of 90 degree to water surface and the position of which was 15 cm above the surface of water. Strength of the illumination given by the lamps in combination was measured to fall in a range of 0.1–8.5 *lux* as recorded in Table 3.

Main experiment No. 3

Using the reflector white lamp of 300 watt at 50 volt alone, this experi-

ment was carried out under an illumination graded from section to section in the tank within a range of 0.2–35 *lux*. The lightness in every section is given in Table 4.

Main experiment No. 4

In this experiment, the reflector white lamp of 300 watt alone was used at 100 volt, the illumination being graded within a range of 2.3–965 *lux*. The values of lightness are shown in Table 5.

Table 5. The pattern of the distribution of fish in Experiment (No. 4.)

Section number	Values of illumination		Number of fish	Correction term	Corrected values	Corrected values adjusted for comparison
	lux	Rank				
1	2.30	1	0	+ 10	10	34
2	2.80	1	0	+ 1	1	25
3	2.60	1	0	+ 2	2	26
4	2.50	1	0	− 24	*−24	0
5	2.60	1	0	− 14	−14	10
6	2.30	1	0	+ 9	9	33
7	3.30	1	0	+ 13	13	37
8	3.70	1	0	− 14	−14	10
9	3.40	1	0	− 7	− 7	17
10	5.40	2	0	− 12	−12	12
11	5.50	2	0	+ 7	7	31
12	5.20	2	0	+ 11	11	35
13	4.20	2	3	+ 16	19	43
14	3.80	1	0	+ 3	3	27
15	3.80	1	5	− 17	−12	12
16	27.50	3	0	− 10	−10	14
17	36.00	3	2	+ 7	9	33
18	22.50	3	0	+ 16	16	40
19	81.00	4	13	+ 13	26	50
20	108.00	4	67	− 1	66	90
21	95.00	4	29	− 7	22	46
22	284.00	5	111	− 16	95	119
23	580.00	6	206	− 18	188	212
24	280.00	5	48	+ 13	61	85
25	350.00	5	6	+ 11	17	41
26	965.00	7	80	+ 6	86	110
27	395.00	5	78	+ 2	80	104
In total			648	0	648	1.296

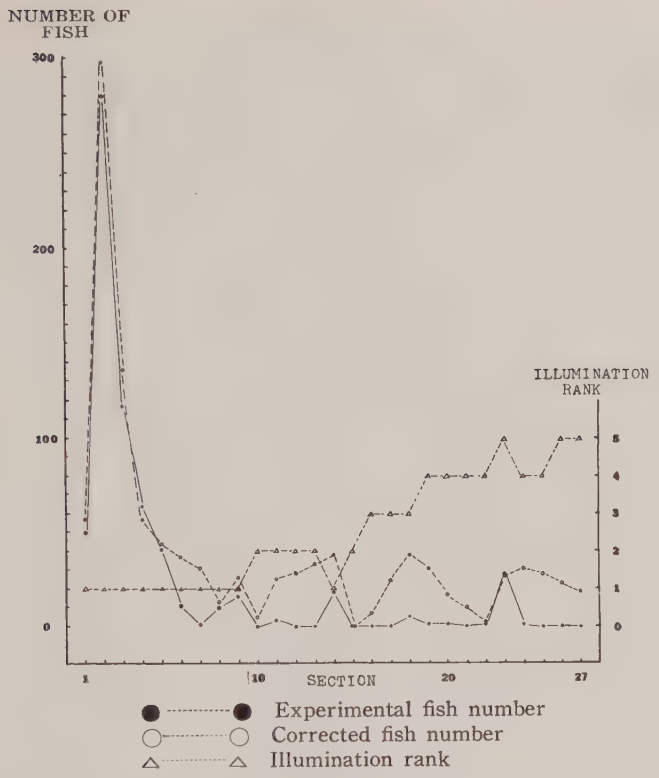

Fig. 5. The pattern of the distribution of fish (Experiment No. 3)

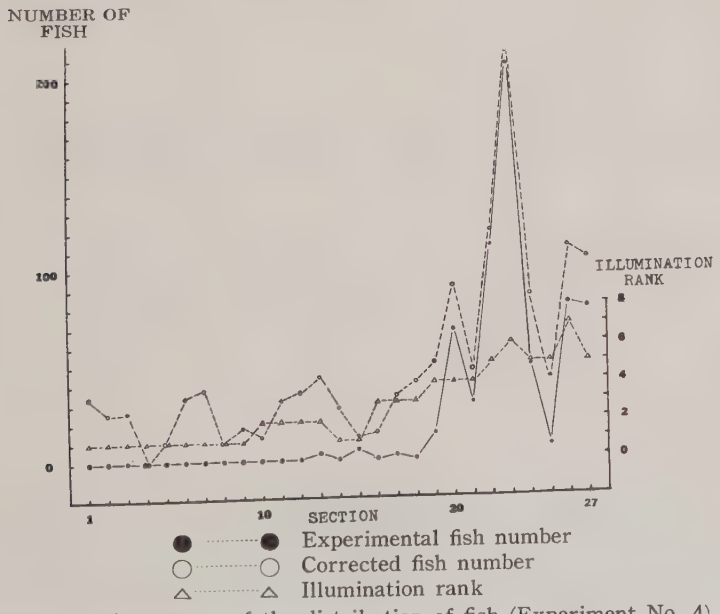

Fig. 6. The pattern of the distribution of fish (Experiment No. 4)

Results of experiment

BEHAVIOR OF THE TEST FISH.

The behaviors of fish observed in said four experiments were as follows:

Table 6. Distribution of fish, for various ranks of illumination (Experiment No. 1.)

Illumination	Rank 1			Rank 2			Rank 3			Rank 4		
	lux	fish	*C. A.	lux	fish	*C. A.	lux	fish	*C. A.	lux	fish	*C.A.
* C.A = Corrected values adjusted for comparison	0.01	0	23	0.02	51	65	0.03	51	66	0.04	7	27
	0.01	4	26	0.02	50	49	0.03	51	40	0.04	114	109
	0.01	18	44	0.02	25	45	0.03	62	61			
	0.01	12	36	0.02	7	23	0.03	27	33			
	0.01	2	31	0.02	3	22	0.03	66	67			
	0.01	3	32	0.02	0	15	0.03	32	28			
	0.01	0	26				0.03	20	23			
	0.01	3	29				0.03	25	37			
	0.01	1	25				0.03	11	17			
							0.03	3	0			
Total		43	272		136	219		348	372		121	136
Means		4.7	30.2		22.6	36.5		34.8	37.2		6.0	6.8
Relative number of fish for a specified rank	%	6.7	27.2	%	20.9	21.9	%	53.8	37.3	%	18.6	13.6
Relative area of specified illumination		33%			22%			37%			8%	

Table 7. The Values, $\frac{\text{Relative number of fish in a specified illumination rank}}{\text{Relative area of specified illumination.}}$ R. F. / A. F.

Illumination / Distribution	Rank 1		Rank 2		Rank 3		Rank 4	
	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison
R. F. / R. A.	0.20	0.82	0.95	1.00	1.45	1.01	2.33	1.70

(1) Under a natural faint light (Experiment No. 1)

The fish scattered at once on removing the confining cylinder and began to swim randomly all around in the tank. In a few minutes most of the test fish gathered in a lighter place to form a group in which every individual fish was found swirling gently. After ten minutes, the group became more condens-

ed than before, comprising quiet individuals. After a half hour, fish began to frequently repeat the formation and resolution of grouping and in the meanwhile most of the fish migrated as they assembled in a group to an upper layer of water, namely, to a place of greater lightness, leaving several individuals behind who continued to swim about gently in a darker place.

(2) Under an illumination given by the lamps of 300 watt and 20 watt both operating at 30 volt (Experiment No. 2)

Table 8. Distribution of fish, for various ranks of illumination (Experiment No. 2.)

Illumination	Rank 1			Rank 2			Rank 3			Rank 4			Rank 5		
	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A
	0.10	45	67	0.31	50	63	1.05	20	45	3.50	4	0	6.60	60	54
	0.10	16	30	0.50	15	17	1.63	17	28	3.40	4	29	8.50	12	30
	0.05	103	91	0.50	4	23	1.23	3	8	3.45	0	23			
	0.10	47	45	0.30	0	28				3.90	2	16			
	0.05	36	57												
* C.A = Corrected values adjusted for comparison	0.04	4	29												
	0.07	59	57												
	0.03	93	98												
	0.05	0	0												
	0.10	11	30												
	0.10	17	40												
	0.10	0	28												
	0.10	10	25												
	0.10	16	11												
Total		457	608		69	131		40	81		10	68		72	84
Means		32.6	43.4		17.2	32.7		13.3	27.0		2.5	17.2		36	42
Relative number of fish for a specified rank	%	70.5	62.5	%	10.6	13.7	%	6.0	8.3	%	1.9	7.0	%	11.0	8.5
Relative area of specified illumination		51.1%			15.0%			11.1%			15.0%			7.8%	

The fish scattered at once on quitting their confinement in the cylinder. They assembled soon afterwards into two groups in a lighter place, in which they swam about actively under the main lamp. In the meanwhile, however, more and more individuals migrated gently towards the supplemental light so as to result in complete migration of the both groups to less illuminated places. After a half hour, most fish were found to have assembled in a less illuminated

Table 9. The values, $\frac{\text{Relative number of fish in a specified illumination rank}}{\text{Relative area of specified illumination}}$ R. F. / R. A.

Illumination / Distribution	Rank 1		Rank 2		Rank 3		Rank 4		Rank 5	
	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison
R. F. / R. A.	1.38	1.22	0.71	0.91	0.54	0.75	0.14	0.47	1.41	1.08

place from which few members were observed to return to the neighborhood of the main light.

In this experiment fish were observed to spend almost all their times in a dark place. The experimenters concerned received from the manner of swimming of test fish a strong impression as follows: (*a*) The fish in group behaved themselves always according to the motion of a certain individual having a style

Table 10. Distribution of fish, for various ranks of illumination (Experiment No. 3.).

Illumination	Rank 1			Rank 2			Rank 3			Rank 4			Rank 5		
	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A
	0.20	50	77	0.41	0	5	2.60	0	7	10.50	1	31	23.5	1	2
	0.20	280	298	0.40	3	27	3.42	0	24	17.60	1	17	39.0	28	27
	0.20	117	136	0.39	0	28	2.60	5	38	10.10	0	10	21.3	1	31
*C.A = Corrected values adjusted for comparison	0.20	64	57	0.30	0	33							26.5	0	28
	0.20	41	44	0.30	0	0							38.1	0	23
	0.15	11	37										35.0	0	19
	0.22	1	31												
	0.26	10	13												
	0.25	16	26												
	0.27	18	38												
Total		608	757		3	93		5	69		2	58		30	130
Means		60.8	75.7		0.6	15.5		1.7	23		0.7	19.3		5	21.7
Relative number of fish for a specified rank	%	94	68.4	%	0.5	8.4	%	0.7	6.2	%	0.2	5.3	%	4.6	11.7
Relative area of specified illumination		37%			18.5%			11.1%			11.1%			22.3%	

Table 11. The values, $\frac{\text{Relative number of fish in a specified illumination Rank}}{\text{Relative area of specified Illumination}}$ R. F. / A. F.

Illumination / Distribution	Rank 1		Rank 2		Rank 3		Rank 4		Rank 5	
	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison
R. F. / R. A.	2.54	1.85	0.027	0.45	0.063	0.56	0.018	0.48	0.2	0.52

of leader. (*b*) One and the same individual behaved as leader throughout whole the course of this experiment, the behaviors of all fish being thus represented by this leader fish.

(3) Under the light emitted from the 300 watt lamp being used at 60 volt (Experiment No. 3).

The fish went dispersed at once on being freed but gathered again by degrees in a lighter place. In the meantime they migrated to a less illuminated place and began to swim about gently along the bottom of the tank. After 20 minutes, most fish were found to have assembled in a dim place, forming a quiet group, while some individuals swam about in an active manner in a lighter place, a few of them jumping occasionally out of water. An individual appearing to be the leader fish was seen swimming always beneath the group of fish. Another single fish was observed far apart from the group, swimming actively in a lighter place as if as a feeler.

(4) In the light from the 300 watt lamp working at 100 volt (Experiment No. 4).

On being made free, the fish scattered instantaneously. But they came gathering again by degrees into a group in a lighter zone of the tank as generally observed in any case of the above Experiments No. 1, No. 2 and No. 3. The layer of water in which test fish were observed swimming about extended from the middle to the bottom of the tank. After a half hour, the group of fish was found to be in a rather diffused state, in which every individual fish swam about apparently at random, a few fish jumping up very often into a domain of the highest illumination. In this experiment any of fish individuals was scarcely observed to go separating from their group, all fish swimming usually in a highly illuminated zone.

The above fact that under certain circumstances fish are actually possible to assemble, in contrast with their behaviors observed in the previous experiments, rather in a place of higher illumination has first been observed in this laboratory with anchovy as test fish. However, even with this fish it seemed as if there existed as regards the strength of illumination a special range within which the fish gathered by preference in a dim place (see Experiments No. 2 and No. 3). The threshold luxes of this range were now estimated as follows by observing the fish behavior under a light source whose illuminating power was made to weaken step by step.

Table 12. Distribution of fish, for various ranks of illumination (Experiment No. 4.)

Illumination	Rank 1			Rank 2			Rank 3			Rank 4			Rank 5			Rank 6			Rank 7		
	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A	lux	fish	*C.A
	2.3	0	34	5.4	0	12	27.5	0	14	81.0	13	50	284	111	119	580	206	212	965	80	110
	2.8	0	25	5.5	0	31	36.0	2	33	108.0	67	90	280	48	85						
	2.6	0	26	5.2	0	35	22.5	0	40	95.0	29	46	350	6	41						
	2.5	0	0	4.2	3	43							395	78	104						
* C.A = Corrected values adjusted for comparison	2.6	0	10																		
	2.3	0	33																		
	3.3	0	37																		
	3.7	0	10																		
	3.4	0	17																		
	3.8	0	27																		
	3.8	5	12																		
Total		5	231		3	121		2	87		109	186		243	349		206	212		80	110
Means		0.45	21.0		0.75	30.2		0.67	29.0		36.3	62		60.8	87.2		206	212		80	110
Relative number of fish for a specified rank	%	0.8	17.8	%	0.5	9.3	%	0.3	6.7	%	16.8	14.3	%	37.5	26.9	%	31.7	16.3	%	12.4	8.7
Relative area of specified illumination		40.6%			14.8%			11.1%			11.1%			14.8%			3.8%			3.8%	

Table 13. The values, $\frac{\text{Relative numders of fish in a specified illumination rank}}{\text{Relative area of specified illumination}}$ F. R. / F. A.

Illumination / Distribution	Rank 1		Rank 2		Rank 3		Rank 4		Rank 5		Rank 6		Rank 7	
	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison	Number of fish	Corrected values adjusted for comparison
R. F. / R. A.	0.02	0.44	0.03	0.63	0.03	0.60	1.51	1.29	2.53	1.82	8.34	4.55	3.26	2.29

It was observed that as the voltage applied to the light source lamp dropped from 100 volt to lower and lower values, more and more individuals of the fish went away from the neighborhood of the light souce, the motion of fish becoming less active. When five minutes elapsed and the voltage applied to the lamp dropped to 20 volt, all fish were found swimming gently in a zone of lower illumination.

Response of Anchovy Distribution to an Illumination

The results directly obtained by counting the number of individuals of the test fish in Expriments No. 1, No. 2, No. 3 and No. 4 are given in Tables 6, 8, 10 and 12, respectively. Tables 7, 9, 11 and 13 have been derived from Tables 6, 8, 10 and 12, respectively.

(1) When the light illuminating the tank was a natural dim light (0.01 *lux*-0.04 *lux* in the water of the tank), a little more individuals of test fish were found staying in a place of 0.03 *lux* than in a place of 0.01 *lux*. The number of fish individuals per unit area (of cross section of a vertical water column) decreases in the order of Illumination-ranks 4>3>2>1. In this case, value of the fish gathering ratio increases with increasing lux (see Table 6 and Table 7).

(2) When the tank was illuminated by a 300 watt lamp and a 20 watt lamp, which operated both at 30 volt, the place most crowded by fish was found in a range of illumination of 0.03–0.1 *lux*, wherein the fish gathering ratio attained to a value as high as 62.5%. In this case, the number of fish found per unit area (in the same meaning as above) decreases, in contrast with the above case, in the order of Illumination-ranks 1>2>3>4, the fish gathering ratio growing larger with the decrease in lux (see Table 8 and Table 9).

(3) When the tank was illuminated by a 300 watt lamp working at 60 volt, the part most crowded by fish was a place that belonged to Illumination-rank 1, wherein the fish gathering raito attained to a value as large as 68.4%. The value of the gathering raito was found to decrease in the order of Illumination-ranks 1>3>5>4>2 (see Table 10 and Table 11).

(4) When a 300 watt lamp working at 100 volt was used as light source fish swimming at first scattered all around in the tank soon formed a group which was observed to move to a more highly illuminated place. However, a group heavily crowded by fish never forms under such a strong illumination as this, as seen from the values of fish gathering ratio given in Table 12 and Table 13.

Conclusion

In laboratorial observations performed under different strengths of illumination and extended each for one hour, anchovy (*Engraulis Japonica*) as test fish showed the behaviors outlined as follows:

(1) Under a faint natural light resembling the twilight (0.01～0.04 *lux*) the fish assembled in a lighter place, swimming in a rather gentle manner.

(2) Under artificial illuminations of moderate strengths (0.03～8.5 *lux* and 0.2～39 *lux*) the fish gathered by preference in a less strongly illuminated place, the motion of their individuals becoming settled down.

(3) Under an artificial very powerful illumination (2.3～965 *lux*) the fish swam about actively by preference in a lighter place.
(4) In the viewpoint of the fish gathering ratio, the tendency of the fish to crowd into a group diminished with increasing lightness within a range of 2.3-963 lux.

Whereas it seems as a general tendency prevalent among carp, mackerel, horse mackerel and saury pike that fish assemble by preference in a dim place, anchovy alone shows an apparent peculiarity of gathering under certain strengths of illumination by preference in a lighter water. The gathering of anchovy observed under a dim natural light may be interpreted rather as a suggestion of that fishes, perhaps irrespective of their species, do not like an extreme darkness. Whether fishes other than anchovy also present the tendency of gathering in a water of extreme lightness remains uncertain, since they have not yet been examined under such a strong illumination as applied in the present study to anchovy.

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STUDY ON THE DISPOSITION OF FISH TOWARDS LIGHT (NO. 6.) COMPARE WITH THE DISPOSITION OF *ENGRAULIS JAPONICUS, DECAPTERUS MUROADSI, TRACEUROUS JAPONICUS* AND SCOMBER JAPONICUS.

Yutaka Imamura and Shoichi Takeuchi

(Received Feb. 15, 1960)

We have known that the disposition of fish towards light was differed a little in compliance with species, throughout the fisheries which had used the light.

In this time, the authors experimented to comare with the dispositions of anchovy (*Engraulis Japonicus*), scad (*Decapterus Muroadsi*), horse mackerel (*Trachurous Japonicus*) and mackerel (*Scomber Japonicus*) towards light.

Arrangement of the experiments

A wooden tank of the following size was used; 4 m in length, a breadth of 0.8 m and a height. of 0.5 m. The inside wall of this tank was painted with grey colour. The amount of water was adjusted to be 35 cm in depth. The tank was

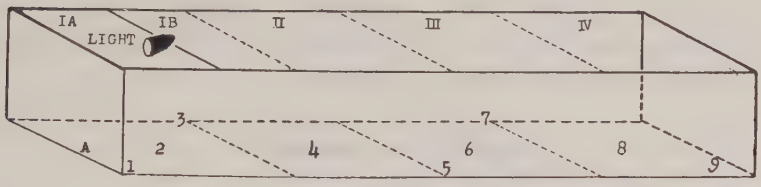


Fig. 1. Arrangement for the experiment

sectioned to four sections equal in area by white line as showing Fig. 1. These sections were named as No. 1, No. 2, No. 3 and No. 4 in order. The section No. 1 was sectioned anew A and B section equal in area and the demarcation of between A and B section was used awooden plate. This demarcation plate had been setting as removable. A reflector white lamp of 300 watt was used as light source and it had been setting on the demarcation line of between A and B section as Fig. 1.

The light was projected at an angle of 45 degree to the water surface and the height of lamp was setted to 25 cm from water-face. Intensity of illumination was controlled by means of a slidax, and while to measure of illumination was done with an under water lux-meter which was kept horizontal at the each measuring point on the tank bottom. The voltage of light was used to 30, 50 and 100 volts through these experiments. When the light had been using to 30, 50 and 100 volts, the illumination values at the every measuring points were shown as Table 1.

Table 1. Values of illumination, at the every measuring point.

Kinds of light / Measuring point	30 volt 300W	50 volt 300 W	100 volt 300 W
	lux	lux	lux
A	60	1.050	7.750
No. 1	33.5	405	2.950
No. 2	2.15	29	62
No. 3	0.15	1.8	11.5
No. 4	0.10	0.85	4.5
No. 5	0.10	0.56	2.9
No. 6	0.10	0.50	2.9
No. 7	0.10	0.40	2.0
No. 8	0.05	0.40	2.0
No. 9	0.05	0.35	1.8

As test fish, 30 individuals of anchovy (average body length 10.1 cm, mean weight 8.47 g), 25 individuals of scad (average body lenght 17.4 cm mean weight 54.2 g), 60 individuals of horse mackerel (average body length 16.5 cm, mean weight 18.5 g) and 16 individuals of mackerel (average body length 25.4 cm, mean weight 189 g) were provided intotal for in these experiments.

The authors were used a fish which had choosen at randomly from these fish. The authors had been using the fish which had raised into the ponds of out door throughout one or two days before to experiment. The water temperature was measured to as 15°–16°C in all the runs. As these experiments were performed in complete absence of any baits.

Method of experiments

Experiment No. 1.

At first, a test fish was kept into the A section of the tank for ten minutes so as to be adapted to dark. After ten minutes, the author switched on the light and then the demarcation plate of between A and B section was removed out from the tank for the fish had been setting to as freely. The experimenters observed to move of fish for twenty minutes. The experiments when were used to 30, 50 and 100 volts as light source were practised two runs with each species.

Experiment No. 2.

The experimenters were practised as well as the experiment No. 1 for to adapte the fish to dark. In this case, the fish was kepted into the A section for 30 minutes after the light was switched on and then the demarcation plate was removed out from this tank. The authors observed to move of test fish as well as the experiment No. 1 for twenty minutes. Intensity of light were used to 30, 50 and 100 volts.

Experiment No. 3.

In this case, the fish was kepted into A section for one hour after the

light had been switched on. This experiment was experimented as well as the former experiments.

In all the runs, the authors made to tracks to swim of the test fish and counted to occupation times of the fish in each section for twenty minutes, after the fish had been freeing.

Result of experiment

The movements of the fish through in these every runs were shown in Table 2,~Table 10. Fig. 2, 3 and 4 were obtained from the Table 2,~Table 10. When the fish was illuminated suddenly with the light, every species had been fidgeted for a while. To fidged of the fish was observed to differ more or less by species through these experiments. When the fish migrated into the section No. 1. the author was presumed that the occupation times of the test fish into No. 1 section had been showing to the phototaxis oneself.

Table 2. Result of Experiment No. 1. The fish was illuminated for 0 min with the 30 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
lux	23.1		0.12		0.11		0.06	
Occupation times / Kinds of fish	Time	Rate	Time	Rate	Time	Rate	Time	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchovy	20.00	100	0	0	0	0	0	0
Scad	8.38	43.1	3.50	19.2	34	2.9	6.58	34.8
Horse mackerel	8.41	43.4	6.46	33.8	1.53	9.4	2.40	13.4
Mackerel	11.44	58.6	2.43	13.6	25	2.1	5.08	25.7

Table 3. Result of Experiment No. 1. The fish was illuminated for 0 min with the 50 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
Lux	201.5		0.9		0.2		0.17	
Occupation times / Kinds of fish	Time	Rate	Tims	Rate	Time	Rate	Time	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchovy	20.00	100	0	0	0	0	0	0
Scad	11.01	55.1	5.10	25.8	2.07	10.6	1.42	8.5
Horse mackerel	11.00	55	1.50	9.1	25	2.0	6.45	33.9
Mackerel	9.31	47.5	3.18	16.5	1.45	6.3	5.57	29.7

Results of experiment No. 1.

The phototaxis of the anchovy was observed as strongly more than the scad, the horse mackerel and the mac kerel as Table 2. But the phototaxis of the anchovy

Table 4. Result of Experiment No. 1. The fish was illuminated for 0 min with 100 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
Lux	2462		4.4		1.63		1.26	
Occupation times / Kinds of fish	Time	Rate	Time	Rath	Time	Rate	Time	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchovy	16.07	80.5	59	4,9	46	4.1	2.08	10.5
Scad	17.05	85.4	45	3.7	10	0.9	2.00	10.0
Horse mackerel	3.15	16.3	14.40	73.3	1.25	7.0	40	3.4
Mackerel	2.59	14.9	9.54	49.5	5.29	29.9	1.17	5.7

Table 5. Result of Experiment No. 2. The fish was illuminated for 30 min with 30 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
Lux	23.1		0.12		0.1		0.06	
Occupation times / Kinds of fish	Time	Rate	Time	Rate	Time	Rate	Times	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchovy	20.00	100	0	0	0	0	0	0
Scad	11.01	55	28	2.3	8.11	40.9	20	1.8
Horse mackerel	7.20	36.6	4.15	21.3	0	0	8.25	42.1
Mackerel	15.30	77.5	3.30	17.5	1.00	5	0	0

Table 6. Result of Experiment No. 2. The fish was illuminated for 30 min with 50 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
Lux	201.5		0.9		0.2		0.17	
Occupation times / Kinds of fish	Time	Rate	Time	Rate	Time	Rate	Time	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchovy	20.00	100	0	0	0	0	0	0
Scad	9.10	45.8	2.25	12.8	1.00	6.0	7.25	35.4
Horse mackerel	20.00	100	0	0	0	0	0	0
Mackerel	7.50	39.1	12.10	60.8	0	0	0	0

was observed a tendency to decrease a little according to fall off the illumination.

When the fish was illuminated with the light of 30 volts and 50 volts, phototaxis of the scad, the horse mackerel and the mackerel had been showing about as half and half between phototaxis and negative phototaxiss of oneself.

Table 7. Result of Experiment No. 2. The fish was illuminated for 30 min with 100 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
Lux	2.462		4.4		1.63		1.26	
Occupation times / Kinds of fish	Time	Rate	Time	Rate	Time	Rate	Time	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchovy	20.00	100	0	0	0	0	0	0
Scad	15.35	77.9	10	0.9	2.50	14.2	1.25	7
Horse mackerel	18.40	93.3	1.04	5.4	16	1.3	0	0
Mackerel	7.07	35.5	1.02	5.1	40	5.0	10.52	54.4

Table 8. Result of Experiment No. 3. The fish was illuminated for one hour with 30 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
Lux	23.1		0.12		0.1		0.06	
Occupation times / Kinds of fish	Time	Rate	Time	Rate	Time	Rate	Time	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchovy	19.20	97	40	3	0	0	0	0
Scad	18.18	91.5	1.42	8.5	0	0	0	0
Horse mackerel	3.32	17.7	3.26	17.1	3.23	16.9	9.39	48.3
Mackerel	40	3.3	0	0	10.00	50.0	9.20	46.7

Table 9. Result of Experiment No. 3. The fish was illuminated for one hour with 50 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
Lux	201.5		0.9		0.2		0.17	
Occupation times / Kinds of fish	Time	Rate	Time	Rate	Time	Rate	Time	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchovy	20.00	100	0	0	0	0	0	0
Scad	19.36	98	24	2	0	0	0	0
Horse mackerel	20.00	100	0	0	0	0	0	0
Mackerel	18.28	92.3	1.32	6.6	0	0	0	0

In the case 100 volts, phototaxis of the scad was risen to 85.4% as well as the anchovy. But phototaxis of the horse mackerel and mackerel were decreased and they were shown to the negative phototaxis oneself.

Result of experiment No. 2.

Phototaxis of the anchovy was shown the heighest values through these

every runs and these values was shown samely to as the experiment No. 1.
Phototaxis of the scad was shown to about as half and half between phototaxis and negative phototaxis oneself through in these runs.
Phototaxis of the horse mackerel was observed to lower values through in these experiments when the light had been used to 30 volts.
But this disposition was risen to the heighest value through in these experiment when the light had been lightning with 50 volts and 100 volts.
Phototaxis of the mackerel was seemed to lower mean value than the other species.

Table 10. Result of Experiment No. 3. The fish was illuminated for one hour with 100 volt 300 watt.

Section	Section No. 1		Section No. 2		Section No. 3		Section No. 4	
Lux	2462		4.4		1.63		1.26	
Occupation times / Kinds of fish	Time	Rate	Time	Rate	Time	Rate	Time	Rate
	m. s	%	m. s	%	m. s	%	m. s	%
Anchouy	15.15	79.2	43	3.5	40	3.5	2.46	13.8
Scad	20.00	100	0	0	0	0	0	0
Horse mackerel	4.47	23.8	3.33	15.2	1.40	8.5	10.30	52.5
Mackerel	3.57	19.7	5.24	27.0	11	1.0	10.28	52.3

Table 11. The fish was illuminated for zero minutes.

Kinds of volt / Kinds of fish	30 volt	50 volt	100 volt
	%	%	%
Anchovy	100	100	80.5
Scad	43.1	55.1	85.4
Horse mackerel	43.4	55	16.3
Mackerel	58.6	47.5	14.9

Table 12. The fish was illuminated for 30 minutes.

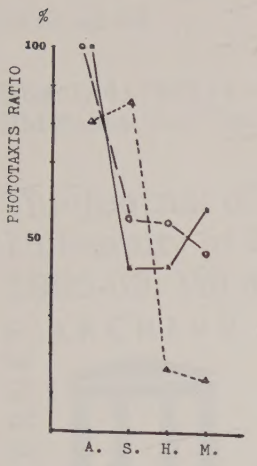
Kinds of volt / Kinds of fish	30 volt	50 volt	100 volt
	%	%	%
Anchovy	100	100	100
Scad	55	45.8	77.9
Horre mackerel	36.6	100	93.3
Mackerel	77.5	39.1	35.5

Results of experiment. No 3.

Through in these experiments, phototaxis of every species was shown a tendency to decrease in generally. But, phototaxis of the scad was looked to increase more than the former experiments and phototaxis of the horse mackerel, the mackerel were shown to as heigher values when the light had been switched on with 50 volts.

Table 13. he fish was illuminated for one hour.

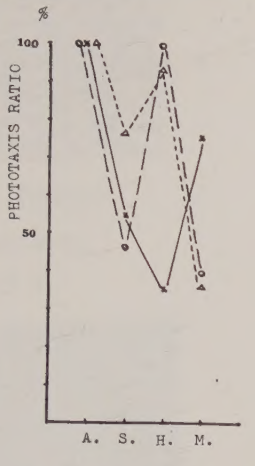
Kinds of volt / Kinds of fish	30 volt	50 volt	100 volt
	%	%	%
Anchovy	97	100	79.2
Scad	91.5	98	100
Horse mackerel	17.7	100	23.8
Mackerel	3.3	92.3	19.7



× —— × 30 volt
○ - - - ○ 50 volt
△ ····· △ 100 volt

A.—Anchovy
S.—Scad
H.—Horse Mackerel
M.—Mackerel

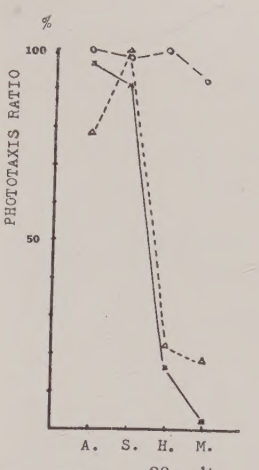
Fig. 2. To illuminative times 0 min.



× —— × 30 volt
○ - - - ○ 50 volt
△ ····· △ 100 volt

A.—Anchovy
S.—Scad
H.—Horse Mackerel
M.—Mackerel

Fig. 3. To illuminative times 30 min.



× —— × 30 volt
○ - - - ○ 50 volt
△ ····· △ 100 volt

A.—Anchovy
S.—Scad
H.—Horse Mackerel
M.—Mackerel

Fig. 4. To illuminative times 60 min.

Conclusion

The author judged to as following through in these experiments.

(1) The anchovy, scad, horse mackerel and mackerel fidgets for a while with the impulse of the moment when the fish was illuminated as directly by the light.

(2) When the fish was illuminated with the light, the fidget-time of the oneself was shorten in due order the anchovy, the scad, the horse mackerel and the mackerel. The author has presumed as following, this order had been showing the strength of the phototaxis oneself.

(3) When the fish had been illuminating for a long time with the illumination of samely strength, phototaxis of fish could not to strengthen according to prolong of the illumination times for oneself.

Phototaxis of the anchovy had been showing a tendency to decrease according to prolong of the illumination-time.

Phototaxis of the scad had been showing a tendency to increase according to prolong of the illumination-time.

Phototaxis of the horse mackerel had been showing a tendency to decrease according to continue of the illumination-time. But the disposition of horse mackerel had been showing a tendency to increase according to prolong of the illumination-time for oneself with heigher illumination and the disposition had been observed a tendency to decrease according to continue of the illumination-time with the heighest illumination.

Phototaxis of the mackerel had been showing a tendency to decrease according to prolong of the illumination-time through in the all cases.